Oligoclonal Repertoire of the CD8αα and the CD8αβ TCR-α/β Murine Intestinal Intraepithelial T Lymphocytes: Evidence for the Random Emergence of T Cells

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Summary

The epithelium of the small intestine in normal euthymic mice contains a large number of intraepithelial lymphocytes (IEL), some of which bear a T cell receptor α/β (TCR-α/β). About half of these TCR-α/β IEL display the CD8αα phenotype and the remaining have the CD8αβ or the CD4 phenotypes. To examine whether TCR-α/β IEL have a TCR-β chain repertoire as diverse as that of TCR-α/β lymph node lymphocytes (LNL), we used a recently described PCR technique that allows a global analysis of the TCR-β chain repertoire. Within any given mouse, the repertoires expressed in both CD8αα and CD8αβ TCR-α/β IEL populations are oligoclonal and nonoverlapping between the two subsets. The clones are largely conserved through the length of the small intestine of the same individual. However, genetically identical individuals raised under indistinguishable environmental conditions display distinct oligoclonal repertoires. Those findings indicate that few cells of CD8αα or of the CD8αβ phenotype are responsible for the repopulation of the intestinal epithelium.

Epithelial cells constitute a natural barrier to environmental aggressions. The intestinal epithelium is exposed to and reacts with a variety of environmental antigens that enter the body through the digestive tract. A large number of intraepithelial lymphocytes (IEL)[1] expressing either TCR-α/β or TCR-γ/δ on their surface are present in the intestinal epithelium (1–5). CD8αβ TCR-α/β IEL develop upon colonization by normal flora, and few TCR-γ/δ IEL, are found in germ-free mice (4, 6). Based upon their CD8 phenotype, murine gut TCR-α/β IEL can be separated into two subpopulations. One is composed of cells expressing the α but not the β chain of the CD8 molecule. These cells express a homodimeric α chain (CD8αα cells) and have been shown to have a thymus-independent origin (4, 7, 8). The second population is composed of cells expressing CD4 or the heterodimeric αβ CD8 molecule (the same as all CD8 lymphocytes found in peripheral lymphoid organs). Evidence has been presented that this population is the progeny of activated T cells migrating from Peyer's patches after antigen stimulation (9–12) but it has also been reported that some of these cells have a thymus-independent origin and may represent a further maturation step of the first population of IEL (8, 13, 14). Recent evidences, indeed, indicate a dual origin for intestinal IEL (15, 16).

PCR analysis of Vβ transcripts in human IEL samples in which cells were not sorted according to their CD8 phenotype, have shown that the TCR-β chain and α chain repertoires of the total IEL population are oligoclonal (17–19). However, with human IEL, there are obvious genetic differences between the samples studied. In addition, environmental differences such as diet or medication might influence the repertoire of the TCR-α/β IEL. The situation is different between human and murine intestines since the former contains ~10% CD8αα IEL and the latter ~50%.

In our study, we tried to overcome those limitations by undertaking a repertoire analysis of the TCR-β chain in IEL from genetically identical mice raised under similar environmental conditions. To ensure an identical environmental, we also studied the TCR-α/β IEL repertoire of two littermate mice kept together until they were studied.

We used a PCR technique (20) to study the repertoire of sorted CD8αα and CD8αβ TCR-α/β IEL subpopulations from different individual mice. We found that the TCR-β chain repertoires of both CD8αα and CD8αβ TCR-α/β

[1] *Abbreviations used in this paper:* DN, double negative; IEL, intraepithelial lymphocytes; LNL, lymph node lymphocytes.

IEL populations are oligoclonal, and that these two T cell populations contain distinct clones. Moreover, individual mice from the same litter display different clonal patterns. Analysis of TCR-β chain repertoire in contiguous sections of the same small intestine revealed that most of the predominant clones are present in adjacent thirds of the intestinal epithelium. The implications of these findings in the development of the intestinal immune system are discussed.

Materials and Methods

Animals

3–6-mo-old (C3H×DBA/2) mice were raised in the animal facilities of the Necker-Enfants Malades Hospital. The mice numbered 2 and 3 were from the same litter.

Preparation of Cell Suspensions and Cell Subsets

IEL were prepared as previously described (9) by scraping and passing through a glass wool column. IEL cells were stained with FITC-labeled anti-CD4 + anti-CD8β together with PE-labeled anti-TCR-α/β, and they were sorted as pure cell suspensions in a FACStar$^{+®}$ (Becton Dickinson & Co., Mountain View, CA). The IEL were then separated into two TCR-α/β populations, one containing the $CD4^-CD8^-$ double negative (DN) IEL (1/5) and the CD8$\alpha\alpha$ IEL (4/5) and the other containing CD4 cells (1/3) and the CD8$\alpha\beta$ cells (2/3). The two sorted populations were referred to henceforth as the CD8$\alpha\alpha$ and CD8$\alpha\beta$ populations, respectively. The numbers of recovered cells were as follows: mouse no. 1, 1.3×10^6 CD8$\alpha\alpha$ IEL, 2.6×10^5 CD8$\alpha\beta$ IEL; mouse no. 2, 3.6×10^5 CD8$\alpha\alpha$ IEL, 0.4×10^5 CD8$\alpha\beta$ IEL; mouse no. 3, 4.2×10^5 CD8$\alpha\alpha$ IEL, 2.6×10^5 CD8$\alpha\beta$ IEL; mouse no. 4, 1.1×10^5 CD8$\alpha\alpha$ IEL, 7.0×10^5 CD8$\alpha\beta$ IEL; mouse no. 6, IEL were prepared from the small intestine cut in three pieces of equal length (upper piece, 8.6×10^5 CD8$\alpha\alpha$ IEL, 6.0×10^5 CD8$\alpha\beta$ IEL; mid piece, 4.5×10^5 CD8$\alpha\alpha$ IEL, 1.9×10^5 CD8$\alpha\beta$ IEL; lower piece, 0.7×10^5 CD8$\alpha\alpha$ IEL, 1×10^5 CD8$\alpha\beta$ IEL; mouse no. 7, 5.2×10^6 total IEL. The lymph node lymphocytes (LNL) were prepared from axillary lymph nodes. They contained $\sim 5 \times 10^6$ cells (with $\sim$20% of B cells, and 80% of T cells) that were directly treated for RNA extraction. As the numbers were relatively low, all the cell preparations were mixed with 5×10^6 to 10^7 P815 mastocytoma cells to avoid excessive loss of material during RNA extractions.

Oligonucleotides

The oligonucleotides specific for Vβ6, Vβ8.1, Vβ8.2, Vβ10, Vβ11, and Vβ14, Cβ and Jβ gene segments that were used to measure the Jβ usage in particular Vβ have been previously described (20).

Specific Activity of Fluorescent Jβ Oligonucleotides

In the run-off reactions, the fluorescence intensity is proportional to the quantity of DNA but depends also on the efficiency of the fluorescent primers. To determine the specific activity of each of the 12 Jβ primers, a plasmid containing one Jβ, one Vβ segment, and part of the Cβ was linearized upstream from the 5′ end of the Vβ segment with the appropriate enzymes. Successive dilutions of the digestion (ranging from 100 ng to 12.6 ng/reaction) was subjected to one cycle of run-off reaction with the corresponding fluorescent Jβ-specific primer in one series of tubes, or with the fluorescent Cβ-specific primer in a parallel series of tubes. One-fifth of the reaction mixture of each dilution was analyzed on the DNA sequencer, as described below. The fluorescent Cβ primer was used as a reference. Fluorescence intensities vs. plasmid concentrations were then plotted. The ratio of the slope of the Jβ with respect to the slope for Cβ ranged from 1 to 3, and was used as a correction factor. Identical procedures were performed for 12 primers labeled with the three fluorophores (Fam, Joe, and Tamra), allowing the relative quantification of the 12 Jβ in the three colors, where the sum of the corrected intensities for the 12 Jβ corresponds to 100% for a given Vβ.

PCR

Conditions of PCR Amplification for CDR3 Length Analyses. Total RNA from suspensions containing a known number of cells was purified in cesium chloride gradients, and were reverse-transcribed into cDNA using a $(dT)_{17}$ primer, and a Reverse-Transcriptase AMV (Boehringer-Mannheim, Mannheim, Germany) along with Rnasin (Promega Corp., Madison, WI). Aliquots of cDNA corresponding to 100–200 ng of total RNA were amplified to saturation in a 50-μl reaction volume of a mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dNTP, 0.01% Triton X-100, 1 U of *Taq* polymerase (Promega Corp.), and 0.25 μM Cβ primer and 0.25 μM of one of the Vβ primers. The detailed protocol is given in (20).

Primer Extension in Run-off Reactions. In this second step, 1 μl of the PCR product was distributed in 12 tubes each containing 8 μl of 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dNTP, 0.01% Triton X-100, 1 U of *Taq* polymerase (Promega Corp.) and 1 μl of 1 μM of one of the 12 fluorescent Jβ primers. The 12 tubes were subjected to 3–10 cycles of primer extension. The use of a fluorescent Cβ primer in the same conditions allowed us to perform a first global analysis (i.e., the sum of the 12 Jβ). One cycle of primer extension starts with a denaturation step of 2 min at 94°C, followed by 1 min at 60°C and 2 min at 72°C, and is stopped after an incubation of 10 min at 72°C. One-tenth of the reaction mixture was loaded on a 6% acrylamide, 8 M urea gel on a DNA Sequencer (model 373A; Applied Biosystems Inc., Foster City, CA). Under these conditions, where only the fluorescent Jβ primer is used, the fluorescence intensity is proportional to the quantity of DNA in each tube. To verify that the number of cycles does not modify the ratio between the 12 Jβ, run-off reactions were performed on the same PCR product, with 1, 3, 5, or 10 cycles, and the relative percentage of each of the 12 Jβ was calculated. These percentages were always identical. The reproducibility of the results was established by analyzing the products of two independent PCR.

Although this technique is a semiquantitative PCR, the values in percentage are corrected for the specific activity of the fluorescent Jβ primers, and therefore, correspond to the real representation of sequences expressing those Jβ segments.

Data Analysis

Size determination of the primer-extension products and peak areas were determined as described (20). Following Kabat et al. (21), the CDR3-like region was taken as encompassing amino acids 95–106.

Direct Sequencing of PCR Products

Vβ–Jβ PCR products were purified and sequenced as described (22).

Results

The TCR-α/β IEL Population Is Likely To Be Oligoclonal, When Compared with Lymph Nodes. We used a previously described PCR-based technology (20, 23) to analyze the β chain repertoire in the gut IEL population and the LNL of individual $C_3H \times DBA/2$ mice ($H\text{-}2^{k/d}$, $Mls\text{-}1^{a}$). Briefly, mRNA extracted from gut samples was converted into cDNA, then amplified to saturation with Vβ- and Cβ-specific primers. Each amplification product was subjected to 3–10 cycles of run-off reaction with each of the 12 fluorescent Jβ primers. The elongated products containing the CDR3 hypervariable region can be resolved by high resolution electrophoresis, resulting in multiple bands corresponding to different CDR3 lengths for each Vβ–Jβ couple under study. Software analysis measured both the length and the area for each band which was then graphically represented as a peak.

Earlier studies with thymus, lymph nodes, and blood lymphocytes of mice have shown that, in each Vβ–Jβ combination, there are five to eight size peaks spaced by three nucleotides corresponding to inframe transcripts (20, 23). The area of each peak is proportional to the quantity of elongated products of this size. Each peak corresponding to a given CDR3-like length contains multiple distinct sequences, but the actual diversity of the sequences within a size peak is so far unknown.

5 out of the 20 Vβ primers were chosen to perform a first analysis on total TCR-α/β IEL and total LNL from the mouse no 7. The results obtained with the Vβ4, Vβ8.1, Vβ13, and Vβ18 primers are shown in Fig. 1. In all the combinations, the LNL cDNA (*dotted lines*) displayed a series of five to seven peaks. In the IEL (*solid lines*), a single peak for Vβ8.1 and Vβ18 (Fig. 1, *B* and *D*), two peaks for Vβ4 (Fig. 1 *A*), and

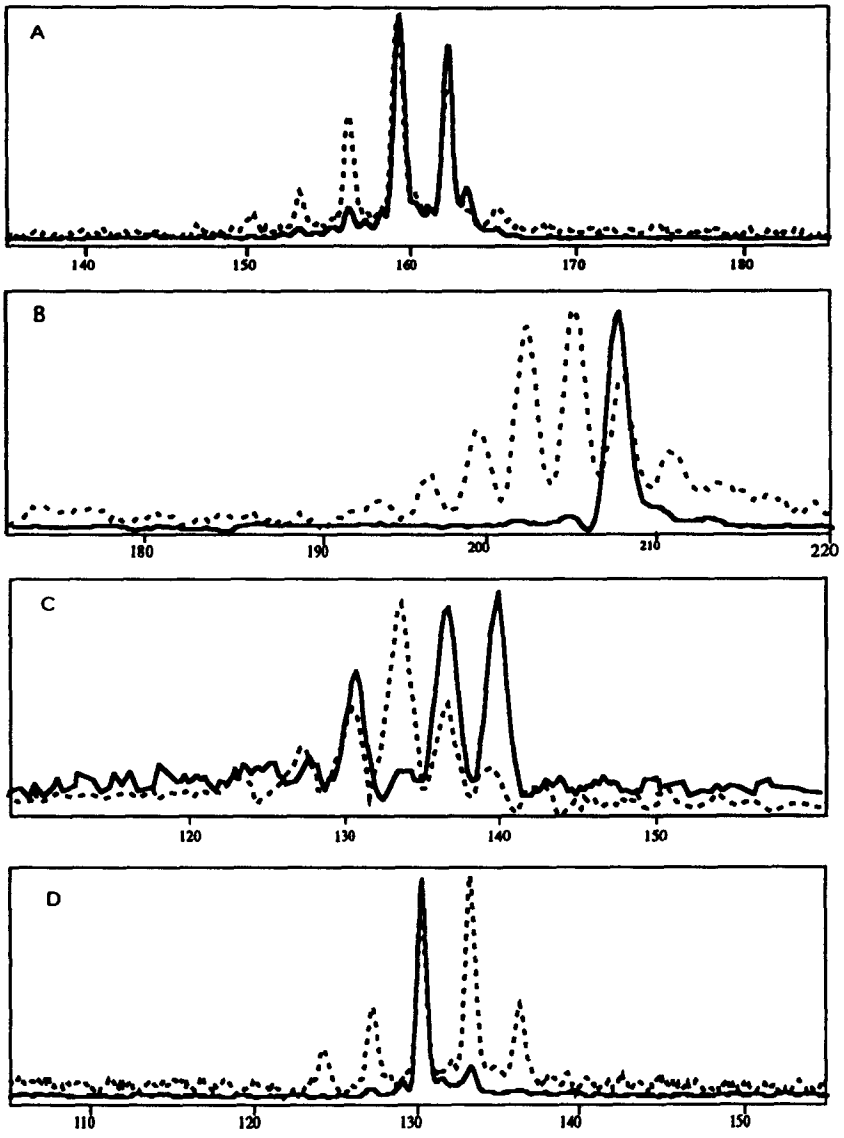

Figure 1. Graphic display of the fluorescent Vβ4–Jβ2.1, Vβ8.1–Jβ2.2, Vβ13–Jβ1.2, and Vβ18–Jβ1.2 run-off products obtained from total IEL and total LNL from the mouse no. 7. After amplification of the cDNA with Vβ4, Vβ8.1, Vβ13, and Vβ18 primers and with Cβ primer, the run-off reactions were performed with Jβ primers labeled with the Fam fluorophore for the IEL (*solid lines*), and with the Joe fluorophore for the lymph nodes (*dotted lines*). The graph represents the intensity of fluorescence in arbitrary units as a function of the size of single-strand DNA fragments in nucleotides.

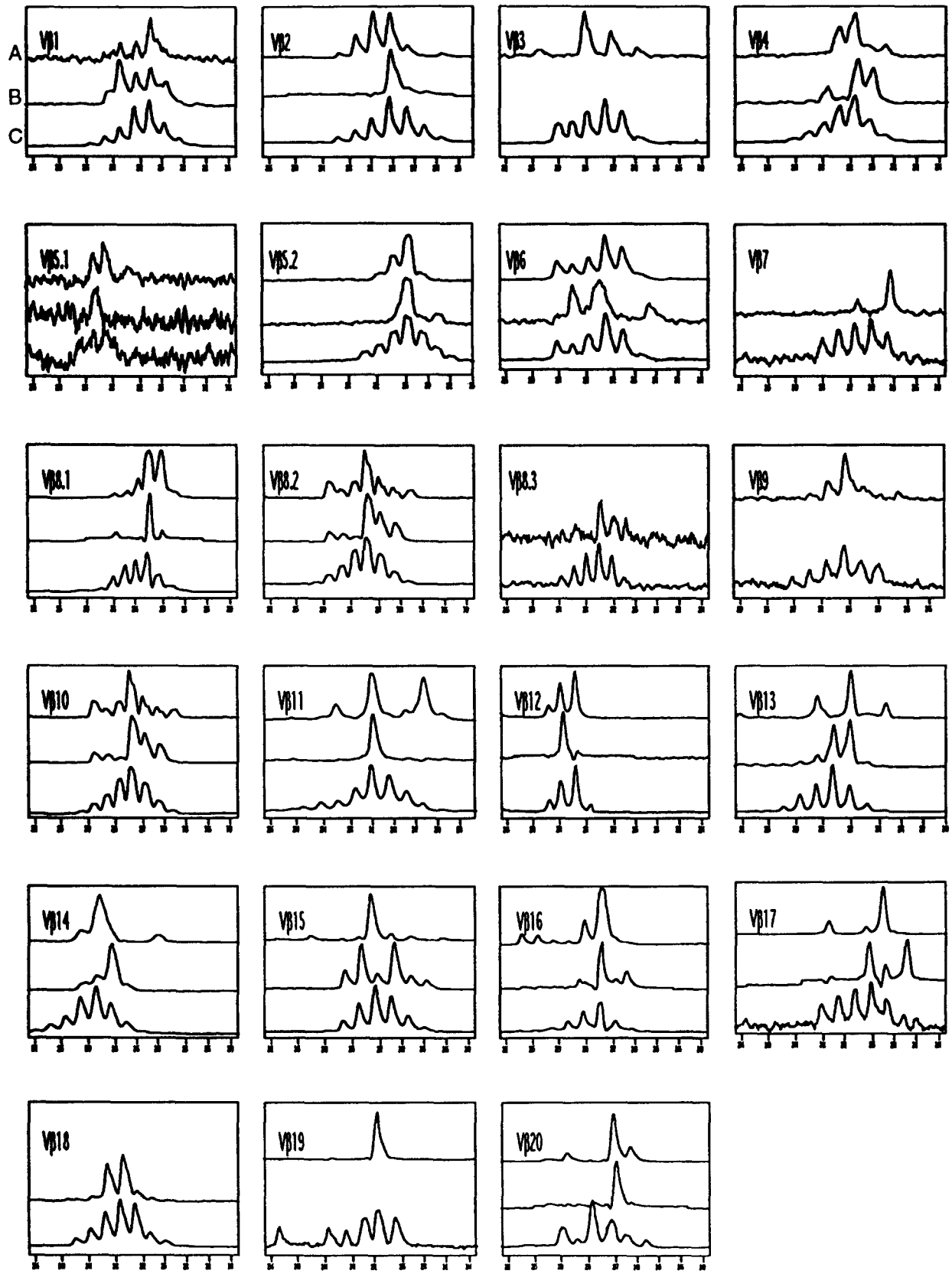

Figure 2. TCR-β chain expression in adult (C3H × DBA/2) F1 CD8$\alpha\alpha$ TCR-α/β IEL (*A*), CD8$\alpha\beta$ TCR-$\alpha\beta$ IEL (*B*), and LNL (*C*). Total RNA was extracted from the LNL and CD8$\alpha\alpha$ and CD8$\alpha\beta$ TCR-α/β IEL populations from mouse no. 2. Each cDNA was synthesized and amplified with the 23 Vβ- and 1 Cβ-specific primers. A run-off reaction using the Cβ-specific fluorescent primer was carried out in parallel on the 23 PCR products. The run-off products were analyzed in a sequencer (model 373A; Applied Biosystems Inc.). CDR3 lengths were calculated as described in Materials and Methods. The graph represents the intensity of fluorescence in arbitrary units as a function of the size of single-strand DNA fragments in nucleotides. In this experimental set up, the relative usage of each Vβ cannot be measured, since the PCR conditions for the 23 Vβ are not quantitative.

three peaks for Vβ13 (Fig. 1 *C*) were observed. Similar results were observed with Vβ9 (data not shown). Size measurements (described in Materials and Methods) showed that the peaks in IEL cDNA had the expected size for inframe transcripts. Three individual mice were studied with the same Vβ primers. It is interesting to note that their IEL all displayed different patterns, in contrast to their lymph nodes, in which more complex but comparable patterns were observed. In previous studies, profiles similar to those observed for IEL have been shown to correspond to the expansion of a limited number of clones (23). Thus, it appeared likely that these IEL populations were oligoclonal and that different oligoclonal expansions had taken place in different individuals. Additional experiments were performed to confirm this point and to analyze the distribution of the expanded clones in different IEL subpopulations of individual mice.

Both IEL Populations Are Oligoclonal. We then fractionated the IEL populations from the gut of five individual mice from either the same or different litters into two populations: the CD8αα IEL and the CD8αβ TCR-α/β IEL (see Materials and Methods). The proportion of CD4 cells in the second population is ~30%; a polyclonal repertoire in these cells would lead to the highest peak in the normal distribution representing 3% of the Vβ–Jβ repertoire. These peaks should be visible in all the repertoire analyses we made. Since this is not the case, it is possible that $CD4^+$ cells have also a oligoclonal repertoire.

We first wanted to know whether all Vβ gene segments are used in both populations of IEL. To this end, we used a modified version of the above approach in which a nested fluorescent Cβ primer, rather each of the 12 Jβ primers, is used as read out in the final run-off reaction after the 23 Vβ–Cβ PCRs. This allows us to determine the sum of the 12 individual reactions yielded by the Jβ primers. Results with the two IEL populations isolated from the mouse no. 2 are shown in Fig. 2. In the CD8αα IEL, 20 out of the 23 Vβ were used, Vβ7, Vβ8.3, and Vβ18 transcripts being undetectable. In the CD8αβ population, 20 out of the 23 Vβ were used as well, but the transcripts from Vβ3, Vβ9, and Vβ19 were not detectable. Contrary to the profiles obtained with LN cells which all displayed the usual gaussian-like distribution of size peaks, each of the CD8αα or CD8αβ IEL profiles was irregular. These results suggested that these Vβ–Cβ profiles were the sum of oligoclonal Vβ–Jβ profiles, a few Vβ distinct in each population being occasionally undetected, presumably by lack of clonal expansions, with no obvious correlation with deletions related to known superantigens (24).

To refine these results, we focused on five Vβ families (Vβ6, Vβ8.1, Vβ11, Vβ8.2, Vβ10), three of which are partially deleted in the periphery of these mice because of their reactivity to endogenous superantigens (Vβ6, Vβ8.1, Vβ11) (reviewed in [24]).

The LNL cDNA displayed five to seven peaks, as shown for Vβ8.1 Vβ8.2, and Vβ10 families of mouse no. 1 in the three-dimensional representation of Fig. 3, while the CD8αα and CD8αβ TCR-α/β IEL displayed a very restricted pat-

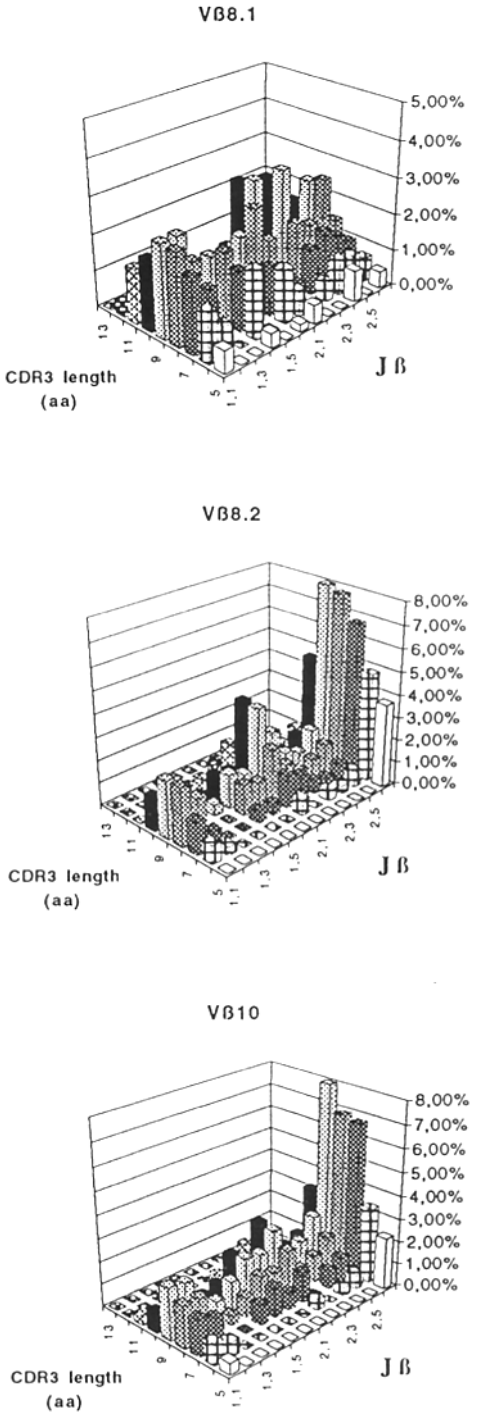

Figure 3. Vβ8.1, Vβ8.2, and Vβ10 T cell repertoire in LNL. Total RNA was extracted from the LNL from mouse no. 1. cDNA was synthesized and amplified with Vβ8.1-, Vβ8.2-, Vβ10-, and Cβ-specific primers. 12 run-off reactions using each of the 12 Jβ-specific fluorescent primers were carried out in parallel on the PCR products. The run-off products were analyzed as described in Fig. 2. CDR3 length were calculated as described in Materials and Methods. The horizontal axes represent the 12 Jβ vs. the CDR3 length in amino acid ranging from 5 to 13. The vertical axis represents the percentage of a given Jβ–CDR3 length combination in the whole Vβ8.1, Vβ8.2, and Vβ10 cell populations. Then, in this experimental set up, the relative usage of each Jβ was measured, since the run-off conditions for the 12 Jβ are identical and the specific activities of the 12 Jβ primers are comparable.

tern. This is illustrated in the three-dimensional representation of Fig. 4, for the analysis of Vβ6, Vβ8.1, and Vβ10 IEL from the mouse no. 1. Fig. 5 shows the results obtained with Vβ11 IEL from three individual mice (nos. 1, 2, and 3), two of which belong to the same litter (mouse no. 2 and mouse no. 3). Our data allow two conclusions to be drawn: (*a*) in the same mouse, the patterns obtained with the two gut TCR-α/β IEL populations are distinct and (*b*) these patterns are different in different individual mice.

It can be seen in Figs. 4 and 5 that the pattern obtained with the IEL subpopulations with certain Vβ–Jβ combinations are, in several cases, restricted to a single major peak. This gave us the opportunity to explore whether such single peaks might correspond to unique Vβ–Dβ–Jβ rearrangements, by performing direct sequencing of the Vβ–Jβ PCR products. In the 16 cases where this was done, a readable sequence was obtained, demonstrating that a single peak corresponds to a homogenous Vβ–Dβ–Jβ rearrangement. The nucleotide sequences are shown in Table 1. As expected, the sequences are in frame. Most of them exhibit N-additions, showing that at the time of Vβ–Dβ–Jβ rearrangement, the terminal deoxynucleotide transferase (TdT) activity was fully present. No obvious amino acid conservation was observed. Furthermore, no obvious differences in the number and the location of deleted nucleotides at the junctions of the Vβ, Dβ, Jβ gene segments could be seen, as displayed in Table 2. When 10 times more cDNA was used in the PCR reaction, no evidence was found for the presence of clones other than the major ones.

These results clearly established that a prominent peak corresponds to a single Vβ–Dβ–Jβ rearrangement and therefore, that the size of the β chain repertoire in murine IEL is much more restricted than in conventional secondary lymphoid organs.

IEL Repertoire Along the Intestinal Epithelium of a Single Mouse. We decided to investigate the extent of variation of the oligoclonal repertoire along the intestine of a single mouse. The small intestine of the mouse was cut into three sections of equal length and, for each segment, the CD8αα IEL and the CD8αβ IEL were separately isolated. The percentages of the dominant peaks in Vβ6, Vβ8.1, Vβ8.2, Vβ10, and Vβ11 families (measured as in the plots of Figs. 3, 4, and 5) along the three intestinal segments are summarized in Table 3. A salient feature of the IEL distribution is that 40% of the peaks in each population are identical in the three segments of intestine, and an additional 44% in two adjacent segments. Only 16% of the predominant peaks were present in only one piece of intestine. We explored whether these predominant peaks correspond to unique Vβ–Dβ–Jβ rearrangements. In the case of two peaks identical in the three pieces of the intestine (one for CD8αα IEL and for CD8αβ IEL), PCR products were directly sequenced, showing identical sequences in the three segments of the small intestine (Table 1).

This demonstrates that some CD8αα and some CD8αβ T cells with the same β chain rearrangement are present throughout the whole length of the small intestine epithelium. Moreover, the clonal pattern of the two TCR-α/β IEL populations is different, indicating that the two populations result from different clonal expansions.

Discussion

In humans, IEL samples from patients with various colorectal diseases or patients undergoing gastric bypass surgery were studied (17, 18). A PCR analysis indicated that an oligoclonal repertoire was expressed in different individuals, and that the repertoire was different for each sample. However, there are obvious genetic differences between individuals and the environmental background might also influence the TCR-α/β IEL repertoire. Such studies, on the contrary, can be conducted in the mouse, as reported here.

We have investigated the diversity of the TCR-β chain repertoire of IEL isolated from littermates and from genetically identical mice raised in the same environment, using a PCR technique to analyze Vβ–Jβ transcripts (20). We first demonstrated that both CD8αα IEL and CD8αβ IEL populations are largely oligoclonal, in contrast to lymph node T cells. It should be noted that the CD8αβ population also contains a minority of $CD4^+$ IEL and that the CD8αα population contains a small proportion of $CD4^-CD8^-$ DN TCR-α/β IEL. Because we were unable to find a background of a polyclonal repertoire even within an amplification of 10 times more cDNA, it is reasonable to think the repertoires of the $CD4^+$ IEL and the $CD4^-CD8^-$ DN IEL would also be oligoclonal. Our evidence relies on the analysis of the length of the hypervariable CDR3-like region of the β chain transcripts in each Vβ–Cβ combination, as well as in a number of different Vβ–Jβ combinations. The global analysis of CDR3-size peaks for the 23 Vβ segments showed an oligoclonal distribution for each of them. For three of the Vβ, we did not find any transcripts, presumably because of the lack of clonal expansion of the cells expressing those Vβ as part of their TCR. The missing Vβ transcripts were not the same in the CD8αα and CD8αβ IEL populations, and did not correlate with the Vβ known to be deleted through the result of interaction with endogenous superantigens.

To analyze in more detail the TCR-β chain repertoire of those two IEL populations, we studied the representation of the Vβ6, Vβ8.1, Vβ8.2, Vβ10, and Vβ11 in combination with all 12 functional Jβ in the LNL and IEL populations. Whereas LNL samples displayed complex patterns as previously demonstrated (20, 23), the IEL showed patterns implying a restricted repertoire. Since we have not analyzed the Vα chain rearrangements, it is possible that T cells having the same Vβ rearrangement could display variable α chains. Nevertheless, a study of human IEL T cell lines has shown an oligoclonality of α chains (17) suggesting that the dominant Vβ rearrangements that we observed correspond to expanded and/or accumulated T cell clones. It should be noted that the overrepresentation of some clones over a background of an otherwise normally diverse repertoire will result in the same graphical patterns as the presence of a few clones in the strict absence of all the others. The absence of detectable

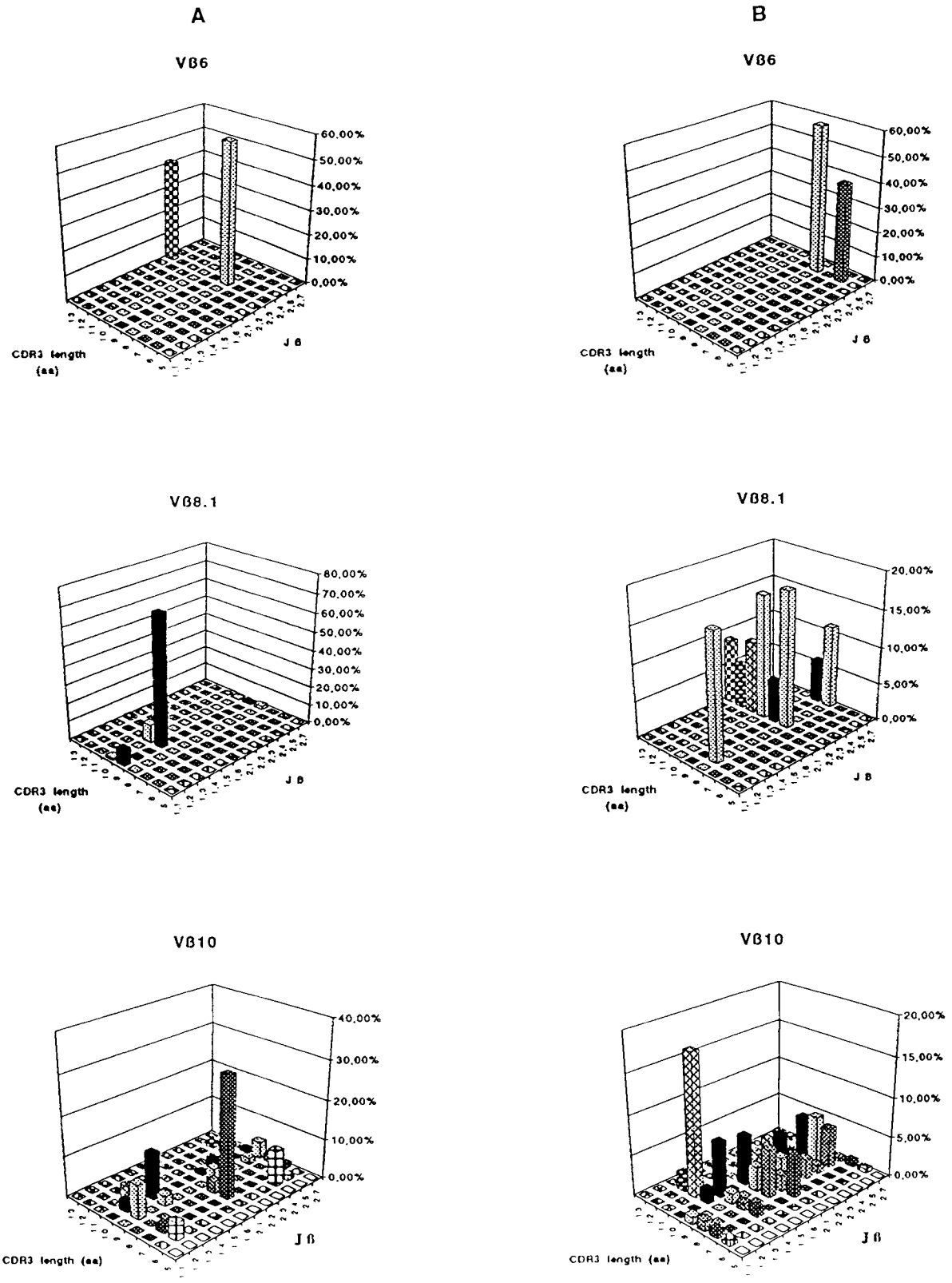

Figure 4. Vβ6, Vβ8.1, and Vβ10 repertoires are oligoclonal in CD8αα and CD8αβ IEL. Total RNA from both IEL populations of the mouse no. 1 were analyzed as described in Fig. 3. *Left panels* (*A*) represent the CD8αα IEL β chain repertoires of the mouse for Vβ6, Vβ8.1, and Vβ10. The *right panels* (*B*) represent its CD8αβ IEL β chain repertoires.

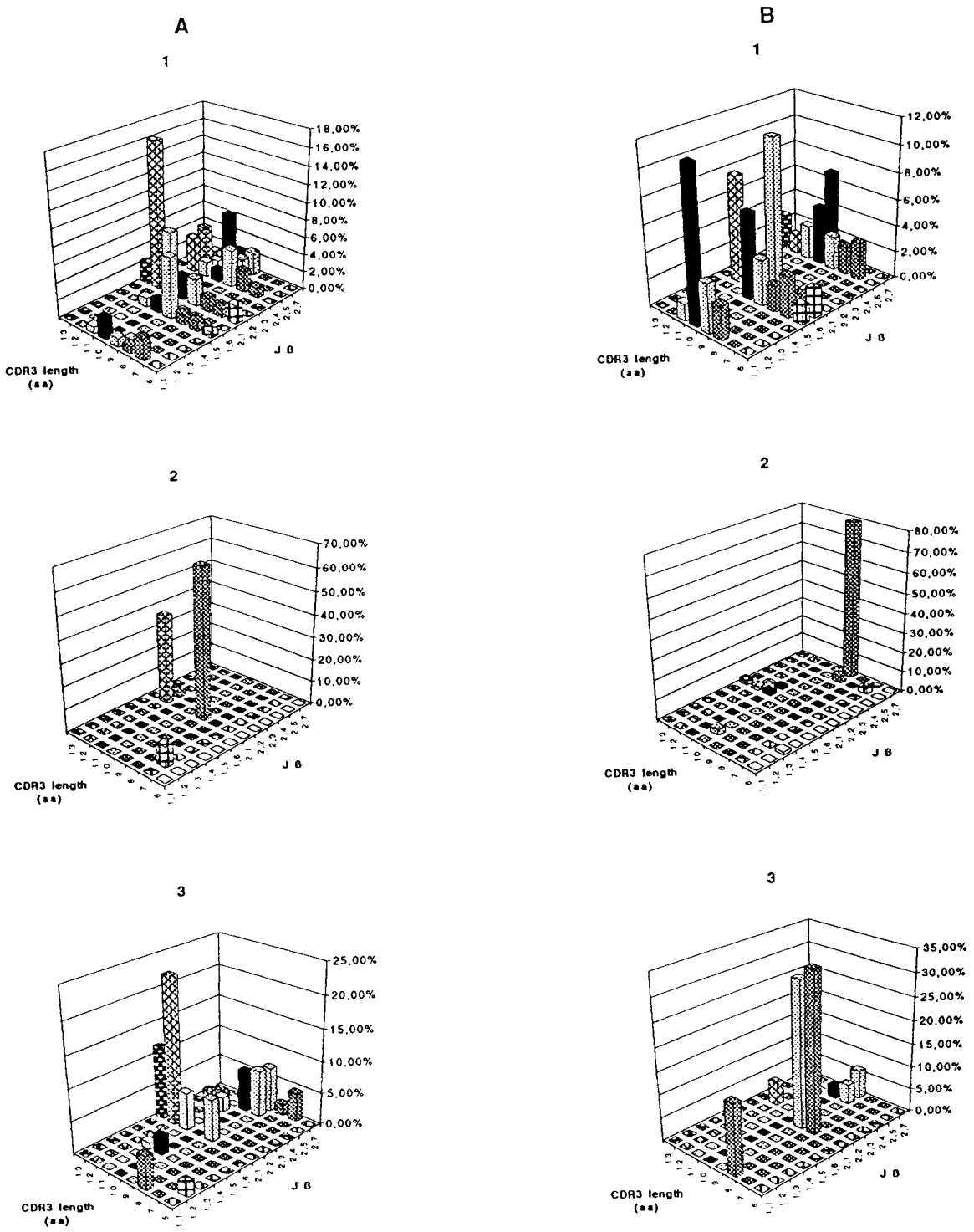

Figure 5. Vβ11 repertoire is oligoclonal in CD8αα and CD8αβ IEL. Total RNA from both IEL populations of the three individual mice (nos. 1, 2, and 3) were analyzed as described in Fig. 3 for LNL cDNA. *Left panels* (*A*) represent the CD8αα IEL β chain repertoires of the three mice (nos. 1, 2, and 3). *Right panels* (*B*) represent their CD8αβ IEL β chain repertoires.

clones other than the major ones, even when 10 times more cDNA was used in the PCR reaction, argues that no diverse normal repertoire exists as a background with respect to the expanded clones.

In the Vβ–Jβ analysis, for each of the six Vβ that we have studied, we found 5–20 peaks in the CD8αα IEL and the CD8αβ gut TCR-α/β IEL subpopulations. This allows us to estimate that each of these populations is composed of

Table 1. *Sequences of Vβ-Dβ-Jβ Junctional Regions from mRNA from CD8αβ and CD8 αα IEL*

. . . Vβ	Dβ	Jβ . . .	Mouse	Population	Sequence	CDR3 length
C A S S I	G G	N Q A P L F				*aa*
TGTGCCAGCAGTAT	AGGGGG	CAACCAGGCTCCGCTTTTT	6*	CD8αα	Vβ06–Dβ1–Jβ1.5	8
C A S S	Q E G G	N Q D T Q Y F				
TGTGCCAGCAGC	CAGGAGGGGGGT	AACCAAGACACCCAGTACTTT	2	CD8αα	Vβ10–Dβ1–Jβ2.5	10
C A S S	W D Q G	N S D Y T				
TGTGCCAGCAGC	TGGGACCAGGGG	AACTCCGACTACACC	3	CD8αα	Vβ10–Dβ1–Jβ1.2	9
C A S S	P D W G G A	N Y A E Q F F				
TGTGCAAGCAGC	CCAGACTGGGGGGGCGCG	AACTATGCTGAGCAGTTCTTC	6	CD8αα	Vβ11–Dβ2–Jβ2.1	12
C A S S I	N R	N S P L				
TGTGCCAGCAGTAT	AAACAGA	AATTCGCCCCTC	4	CD8αα	Vβ06–Dβ1–Jβ1.6	8
C A S S I	S	Y E Q Y F				
TGTGCCAGCAGTAT	CAG	CTATGAACAGTACTTC	2	CD8αβ	Vβ06–Dβ1–Jβ2.7	6
C A S S I	T G E	Y S P L				
TGTGCCAGCAGTAT	CACAGGGGAGT	ATTCGCCCCTC	3	CD8αβ	Vβ06–Dβ1–Jβ1.6	9
C A S S	G T G G	Y A E Q				
TGTGCCAGCAGT	GGGACAGGGGG	CTATGCTGAGCAG	2	CD8αβ	Vβ82–Dβ1–Jβ2.1	9
C A S S D	G G E	G Q L				
TGTGCCAGCAGTGATG	GAGGGGAA	GGGCAGCTC	3	CD8αβ	Vβ82–Dβ1–Jβ2.2	8
C A S S	W S G G	Q D T Q Y F				
TGTGCCAGCAGC	TGGAgTGGGGGT	CAAGACACCCAGTACTTT	3	CD8αβ	Vβ10–Dβ2–Jβ2.5	9
C A S S	T G T G V	Y E Q Y F				
TGTGCAAGCAGC	ACCGGGACTGGGGT	CTATGAACAGTACTTC	2	CD8αβ	Vβ11–Dβ2–Jβ2.7‡	9
C A S S	H P	T E V F F				
TGTGCAAGCAGC	CACCC	CACAGAAGTCTTCTTT	6*	CD8αβ	Vβ11–Dβ?–Jβ1.1	6

D region sequence that cannot be definitively assigned to Dβ1 or Dβ2 is assigned by a question mark. The 3′ end of the Vβ6 and Vβ11 sequences are the consensus from the sequences that we found in the literature and therefore are only an estimate of the germline sequences. Minimal estimated N-region nucleotides were defined as any nucleotides that could not have been accounted for any of the germline gene segments. The CDR3-like region was taken as encompassing amino acids 95–106. Mouse no. 6: sequences made with RNA extracted from a small intestine cut in three sections of equal length.

* The same sequences were performed and found in the three sections of small intestine.

‡ The peak corresponding to this sequence is represented on the Fig. 5 *B*, panel *2*.

Table 2. *Analysis of the TCR-β Chain Sequences of CD8β⁺ and CD8β⁻ Gut IEL*

Mouse	Gut IEL population	TCR-β chain sequence	CDR3 length (aa)	Exo 3′ V* (nt)	N V-D (nt)	Exo 5′ D* (nt)	D length (nt)	Exo 3′ D* (nt)	N D-J (nt)	Exo 5′ J* (nt)
3	CD8β⁺	Vβ06-Dβ1-Jβ1.6	9	1	1	3	7	2	3	8
2	CD8β⁺	Vβ06-Dβ1-Jβ2.7	6	1	0	4	3	5	0	5
3	CD8β⁺	Vβ10-Dβ2-Jβ2.5	9	0	1	1	10	3	1	3
6 (up-md-lw)	CD8β⁺	Vβ11-Dβ?-Jβ1.1	6	0	1	3	2	7	2	4
2	CD8β⁺	Vβ11-Dβ2-Jβ2.7	9	0	3	0	10	4	1	5
2	CD8β⁺	Vβ82-Dβ1-Jβ2.1	9	4	0	0	11	1	0	3
3	CD8β⁺	Vβ82-Dβ1-Jβ2.2	8	0	1	5	5	2	2	8
6 (up-md-lw)	CD8β⁻	Vβ06-Dβ1-Jβ1.5	8	1	0	5	6	1	0	3
4	CD8β⁻	Vβ06-Dβ1-Jβ1.6	8	0	1	3	4	5	1	7
3	CD8β⁻	Vβ10-Dβ1-Jβ1.2	9	0	1	4	6	2	0	2
2	CD8β⁻	Vβ10-Dβ1-Jβ2.5	10	0	4	5	6	1	2	0
6 (lw)	CD8β⁻	Vβ11-Dβ2-Jβ2.1	12	0	3	2	12	0	3	1
		mean CD8β⁺ IEL	8.00	0.86	1.00	2.29	6.86	3.43	1.29	5.14
		SD	1.41	1.46	1.00	1.98	3.63	2.07	1.11	2.12
		mean CD8β⁻ IEL	9.40	0.20	1.80	3.80	6.80	1.80	1.20	2.60
		SD	1.67	0.45	1.64	1.30	3.03	1.92	1.30	2.70
		mean CD8β⁺ and CD8β⁻	8.58	0.58	1.33	2.92	6.83	2.75	1.25	4.08
		SD	1.62	1.16	1.30	1.83	3.24	2.09	1.14	2.61

D region sequence that cannot be definitively assigned to Dβ1 or Dβ2 is indicated by a question mark. CDR3-like region was taken as encompassing amino acids 95–106. Minimal estimated N-region nucleotides that could not have been accounted for by any of the germline gene segments.

* Number of nucleotides digested by exonucleases at the 5′ or 3′ ends of Vβ, Dβ, and Jβ gene segments.

Table 3. *Representativity of the Dominant Clones in the CD8αα and CD8αβ IEL in the Three Sections of the Small Intestine of a Single Mouse*

			Section of intestine (Percentage in the given Vβ)					
			CD8αα IEL			CD8αβ IEL		
Vβ	Jβ	CDR3 length	up	md	lw	up	md	lw
		aa						
6	1.1	8	–	12	19	–	–	–
6	1.5	8	42*	28*	65*	–	–	–
6	2.7	8	31	35	–	18	60	–
6	2.7	9	–	–	–	–	40	–
8.1	1.3	10	–	–	–	7	15	12
8.1	2.1	9	2.5	3	–	29	57	–
8.1	2.7	6	23	23	4.8	–	–	–
8.1	2.7	8	21	17	18	–	–	–
8.2	1.4	10	12	13	–	–	–	–
8.2	2.1	6	–	–	47	–	–	–
8.2	2.1	9	31	15	53	93	90	21
10	1.4	8	–	–	–	33	30	–
10	2.2	9	65	60	53	–	–	–
10	2.7	7	3	7	–	23	40	–
10	2.7	8	8	5	23	22	–	88
11	1.1	6	2	–	–	27*	45*	20*
11	2.1	8	–	–	3	10	–	23
11	2.1	12	39	41	12	–	–	–

Representation of the dominant peaks according to their CDR3 length within CD8αα and CD8αβ IEL populations in the three sections of intestine of the mouse no. 6: the upper section (*up*), the mediane section (*md*), and the lower section (*lw*). The values represent the percentage of each dominant peak within the given Vβ–Jβ combination.
–, under detection limit.
* PCR products directly sequenced (Table 1).

several hundred dominant clones, as compared with hundreds of thousands of clones in lymph node and splenic T cell populations. As an evaluation of the number of T cells in the intestine has indicated about 50×10^6 cells (25), our results imply that some of the IEL clones have either undergone considerable expansion or have been selectively accumulated in the gut.

Three of the Vβ that we studied in greater detail (Vβ6, Vβ8.1, and Vβ11) are deleted by Mls-1^a in the thymus of $(C_3H \times DBA/2)F_1$ mice (26). This did not prevent us from observing complex size patterns of CDR3 lengths in the lymph node samples. Mls-1^a–induced deletions are not complete, and our data indicate no obvious selection or oligoclonality within the T cells that escaped clonal deletion, in agreement with results from others (27).

The above data thus confirm the observation made in a total human IEL population, which showed that the IEL repertoire is oligoclonal (17, 18). Our results lead to several new conclusions since we analyzed sorted populations from genetically identical animals. One is that TCR-α/β IEL, like TCR-γ/δ IEL (5, 28, 29), undergo TCR rearrangements under conditions where the TdT enzyme is active (30) as shown by the presence of N-additions in most β chains sequenced: thus both populations of TCR-α/β IEL are not of embryonic type. The second is that both the CD8αα IEL and the CD8αβ TCR-α/β IEL populations are oligoclonal, but do not share the same dominant clones. The third unexpected conclusion is that individual inbred mice, even from the same litter and maintained in similar environmental conditions, display distinct and apparently nonoverlapping oligoclonal repertoires of both CD8αα IEL and CD8αβ TCR-α/β IEL. Finally, the fact that about 80% of the dominant clones were found in two or three contiguous pieces of small intestine, indicates that the corresponding T cells were capable of migrating along large portions of the small intestine or to recirculate.

What are the mechanisms by which such oligoclonal reper-

toires are established? TCR-α/β IEL are virtually absent in axenic mice (4, 6) and in mice in which the β_2-microglobulin gene has been disrupted, showing that colonization by normal flora and class I MHC molecules play an important role in the selection and/or the maintenance of TCR-α/β IEL (31, 32). Furthermore, in male transgenic mice expressing the TCR specific for the H-Y antigen, the CD8$\alpha\alpha$ IEL are present in the gut (14, 33). These data clearly establish that the emergence of TCR-α/β IEL is antigen driven. It may be argued that in spite of the genetic and environmental homogeneity, the antigens that drive IEL expansion may be completely different in individual mice. The intestinal flora might develop differently in distinct animals, even raised in the same cage. However, it is known that the gastrointestinal flora in a normal adult animal consists of autochthonous (ubiquitous in all members of a community) microbial species that colonize their habitat natively. At birth, the intestines are sterile. The colonization by indigenous flora of the gastrointestinal tract is complete at ~3 wk of age, just after weaning (34). This correlates with the time where TCR-α/β IEL appear in large number in the gut (4). At any given time, allochthonous (accidentally acquired) bacteria may be temporary found in the gastrointestinal canal but they do not colonize it except under abnormal circumstances (35).

The case of the CD8$\alpha\beta$ IEL may be considered first, since more is known about the origin and traffic of at least part of these cells. This population contains the progeny of peripheral thymus-dependent cells, activated by antigens in the Peyer's patches and migrating through the thoracic duct lymph to reach the blood and then to seed the whole length of the gut mucosa, as the result of their special gut-homing properties (9–12, 15). Transfer experiments have indeed shown that T cell blasts obtained from the thoracic duct lymph, labeled in vitro and injected intravenously, are recovered in recipient mice in part as labeled IEL present at all levels of the small bowel. These IEL apparently undergo terminal differentiation, since there is no evidence that they recirculate, at least on a detectable scale. In contrast, small noncycling T lymphocytes do not detectably display, after cell transfer, this property of becoming gut IEL, and recirculate through the lymphoid system (9–12). The oligoclonality of those IEL which are the progeny of blasts antigenically stimulated in the Peyer's patches, and the differences of these oligoclonal repertoires among different inbred mice, in spite of very comparable antigenic exposure, is intriguing. One explanation might be that, at a given time, only a fraction of the potentially responsive circulating T lymphocytes, i.e., of the total lymphocytic population bearing the TCR repertoire corresponding to these common antigens, may be present in the Peyer's patches and undergo stimulation followed by blastic transformation. This would explain the oligoclonality of these IEL which are the terminally differentiated progeny of such blasts; the random nature, in time, of the fraction of the appropriate T cell repertoire that is stimulated would explain the differences observed between mice. On the other hand, the observation that identical clones can be observed at different levels of the small bowel is not surprising, since it reflects the peculiar cell traffic which gives rise to this population. The large size of the oligoclonal pool of these cells would thus correspond to an expansion taking place outside of the epithelium; indeed a large clonal expansion is not likely to be able to take place in situ, since IEL are dispersed and squeezed between epithelial cells that follow a rapid migration from the base or the top of the villosities where they are shed in the gut lumen.

How could the oligoclonality of the CD8$\alpha\alpha$ IEL population and the presence of identical clones in different segments of the gut be explained? The gut epithelium has been shown to initiate and regulate T cell development from bone marrow precursors (4, 8, 13), although thymic factors appear to be required for full expansion (36). Precursors of the CD8$\alpha\alpha$ IEL could enter the gut and rearrange their TCR gene segments in situ, within the epithelium (37), cells bearing appropriate TCR would then be selected by antigen recognition. Thus, in contrast to the CD8$\alpha\beta$ IEL, CD8$\alpha\alpha$ IEL may not be generated from a preexisting pool of mature, long-lived lymphocytes with a wide antigenic repertoire, but from local random recombinatorial, events giving rise to a limited repertoire: such a repertoire would be expected to be distinct in different individuals, and also to change in a given individual if no or few long-lived cells are generated locally (it is known that most IEL are short lived). However, expansion of the emerging clones and their widespread distribution in the gut would require, in this hypothesis, that these cells undergo a traffic taking place outside of the gut epithelium. There is evidence for this possibility, since lymphocytes bearing the $\alpha E\beta 7$ integrin (CD103) characteristic of IEL are found in the blood (38), but further investigations are obviously required.

At any rate, the present observations, and previous ones made in humans and mice, raise the question of whether the TCR specificity of CD8$\alpha\alpha$ and CD8$\alpha\beta$ IEL is relevant to their function. The IEL compartment might be filled up by T cells that have been activated by certain antigenic stimuli. The function of at least some of these IEL might then be independent of the specificity of their TCR. Further work is needed to clarify this issue.

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