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

By Armelle Regnault,* Ana Cumano,* Pierre Vassalli,§ Delphine Guy-Grand,† and Philippe Kourilsky*

From *Unité de Biologie Moléculaire du Gène-U.277 INSERM, Institut Pasteur, 75724 Paris Cédex 15; and the †INSERM Unité 132, CHU Necker-Enfants Malades, 75743 Paris Cédex 15, France; and the §Département de Pathologie, Centre Médical Universitaire, CH-1211 Genève 4, Switzerland

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 oligodonal 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 oligodonal 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) 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 oligodonal (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 environment, 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
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 x 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®. The IEL were then separated into two TCR-α/β populations, one containing the CD4+CD8β double negative (DN) IEL (1/5) and the CD8αβ IEL (4/5) and the other containing CD4 cells (1/3) and the CD8αβ cells (2/3). The two sorted populations were referred to henceforth as the CD8αβ and CD8αβ populations, respectively. The numbers of recovered cells were as follows: mouse no. 1, 1.3 × 10^6 CD8αβ IEL; 2.6 × 10^6 CD8αβ IEL; mouse no. 2, 3.6 × 10^6 CD8αβ IEL; 0.4 × 10^6 CD8β IEL; mouse no. 3, 4.2 × 10^6 CD8αβ IEL; 2.6 × 10^6 CD8β IEL; mouse no. 4, 1.1 × 10^6 CD8αβ IEL; 7.0 × 10^5 CD8β IEL; mouse no. 6, IEL were prepared from the small intestine cut in three pieces of equal length (upper piece, 8.6 × 10^5 CD8αβ IEL; 6.0 × 10^5 CD8αβ IEL; mid piece, 4.5 × 10^6 CD8αβ IEL; 1.9 × 10^6 CD8β IEL; lower piece, 0.7 × 10^5 CD8αβ IEL; 1.0 × 10^5 CD8β IEL; mouse no. 7, 5.2 × 10^5 total IEL. The lymph node lymphocytes (LNL) were prepared from axillary lymph nodes. They contained ~5 × 10^5 cells (with ~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 × 10^5 to 10^6 P815 mastocytoma cells to avoid excessive loss of material during RNA extractions.

Oligonucleotides

The oligonucleotides specific for VB6, VB8.1, VB8.2, VB10, VB11, and VB14, CB and JB gene segments that were used to measure the JB usage in particular VB have been previously described (20).

Specific Activity of Fluorescent JB 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 JB primers, a plasmid containing one JB, one VB segment, and part of the CB was linearized upstream from the 5’ end of the VB 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 CB-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 CB primer was used as a reference. Fluorescence intensities vs. plasmid concentrations were then plotted. The ratio of the slope of the JB with respect to the slope for CB 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, Joc, and Tamra), allowing the relative quantification of the 12 JB in the three colors, where the sum of the corrected intensities for the 12 JB corresponds to 100% for a given VB.

PCR

Conditions of PCR Amplification for CDR3 Length Analyzes. 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)12 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 MgCl2, 0.01% gelatin, 200 μM dNTP, 0.01% Triton X-100, 1 U of Taq polymerase (Promega Corp.), and 0.25 μM CB primer and 0.25 μM of one of the VB 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 MgCl2, 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 JB primers. The 12 tubes were subjected to 3–10 cycles of primer extension. The use of a fluorescent CB primer in the same conditions allowed us to perform a first global analysis (i.e., the sum of the 12 JB), 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 JB 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 JB, 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 JB 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 JB primers, and therefore, correspond to the real representation of sequences expressing those JB 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

VB-JB 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 C3H×DBA/2 mice (H-2k/d, Mls-1). 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 CD8αα TCR-α/β IEL (A), CD8αβ TCR-αβ IEL (B), and LNL (C). Total RNA was extracted from the LNL and CD8αα and CD8αβ 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 LN 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-
tern. This is illustrated in the three-dimensional representation of Fig. 4, for the analysis of $V_{\beta}6$, $V_{\beta}8.1$, and $V_{\beta}10$ IEL from the mouse no. 1. Fig. 5 shows the results obtained with $V_{\beta}11$ 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-$\alpha/\beta$ 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 $V_{\beta}-J_{\beta}$ 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 $V_{\beta}-D_{\beta}-J_{\beta}$ rearrangements, by performing direct sequencing of the $V_{\beta}-J_{\beta}$ PCR products. In the 16 cases where this was done, a readable sequence was obtained, demonstrating that a single peak corresponds to a homogenous $V_{\beta}-D_{\beta}-J_{\beta}$ 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 $V_{\beta}-D_{\beta}-J_{\beta}$ 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 $V_{\beta}$, $D_{\beta}$, $J_{\beta}$ 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 $V_{\beta}-D_{\beta}-J_{\beta}$ rearrangement and therefore, that the size of the $\beta$ 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 CD8$^{+}$ T and the CD8$^{\alpha}$ IEL were separately isolated. The percentages of the dominant peaks in $V_{\beta}6$, $V_{\beta}8.1$, $V_{\beta}8.2$, $V_{\beta}10$, and $V_{\beta}11$ 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 $V_{\beta}-D_{\beta}-J_{\beta}$ rearrangements. In the case of two peaks identical in the three pieces of the intestine (one for CD8$^{\alpha}$ IEL and for CD8$^{\alpha/\beta}$ IEL), PCR products were directly sequenced, showing identical sequences in the three segments of the small intestine (Table 1).

This demonstrates that some CD8$^{\alpha}$ and some CD8$^{\alpha/\beta}$ T cells with the same $\beta$ chain rearrangement are present throughout the whole length of the small intestine epithelium. Moreover, the clonal pattern of the two TCR-$\alpha/\beta$ 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-$\alpha/\beta$ IEL repertoire. Such studies, on the contrary, can be conducted in the mouse, as reported here.

We have investigated the diversity of the TCR-$\beta$ chain repertoire of IEL isolated from littermates and from genetically identical mice raised in the same environment, using a PCR technique to analyze $V_{\beta}-J_{\beta}$ transcripts (20). We first demonstrated that both CD8$^{\alpha/\beta}$ and CD8$^{\alpha}$ IEL populations are largely oligoclonal, in contrast to lymph node T cells. It should be noted that the CD8$^{\alpha}$ population also contains a minority of CD4$^{+}$ IEL and that the CD8$^{\alpha/\beta}$ population contains a small proportion of CD4$^{+}$ CD8$^{-}$ DN TCR-$\alpha/\beta$ 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 $\beta$ chain transcripts in each $V_{\beta}-C_{\beta}$ combination, as well as in a number of different $V_{\beta}-J_{\beta}$ combinations. The global analysis of CDR3-size peaks for the 23 $V_{\beta}$ segments showed an oligoclonal distribution for each of them. For three of the $V_{\beta}$, we did not find any transcripts, presumably because of the lack of clonal expansion of the cells expressing those $V_{\beta}$ as part of their TCR. The missing $V_{\beta}$ transcripts were not the same in the CD8$^{\alpha}$ and CD8$^{\alpha/\beta}$ IEL populations, and did not correlate with the $V_{\beta}$ known to be deleted through the result of interaction with endogenous superantigens.

To analyze in more detail the TCR-$\beta$ chain repertoire of those two IEL populations, we studied the representation of the $V_{\beta}6$, $V_{\beta}8.1$, $V_{\beta}8.2$, $V_{\beta}10$, and $V_{\beta}11$ in combination with all 12 functional $\beta$ 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 $V_{\alpha}$ chain rearrangements, it is possible that T cells having the same $V_{\beta}$ rearrangement could display variable $\alpha$ chains. Nevertheless, a study of human IEL T cell lines has shown an oligoclonality of $\alpha$ chain (17) suggesting that the dominant $V_{\beta}$ 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.

Regnault et al.
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.

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 \( \nu\beta-D\beta-J\beta \) Junctional Regions from mRNA from CD8\( \alpha \beta \) and CD8 \( \alpha \alpha \) IEL

| ... \( \nu\beta \) | \( D\beta \) | ... \( J\beta \) | Mouse | Population | Sequence | CDR3 length |
|-------------------|-------------|----------------|-------|------------|----------|-------------|
| CASSI             | GGGGGGG     | NQAPLFF       |       |            |          | aa          |
| TGTGCCAGCAGCAT    | AGGGGGG     | CAACCAAGCTCGCATTTC | 6\*  | CD8\( \alpha \) | \( \nu\beta 06-D\beta 1-J\beta 1.5 \) | 8            |
| CASS              | QEGGGG      | NQDQTQYF      |       |            |          |             |
| TGTGCCAGCAGC      | CAGGGAGGGG  | AAACAAAGACACACACTTTT | 2    | CD8\( \alpha \) | \( \nu\beta 10-D\beta 1-J\beta 2.5 \) | 10           |
| CASS              | WDQQG       | NSDYT         |       |            |          |             |
| TGTGCCAGCAGC      | TGGACCAGGGG | AACTCCGACTACACC | 3    | CD8\( \alpha \) | \( \nu\beta 10-D\beta 1-J\beta 1.2 \) | 9            |
| CASS              | PDDWGGAGA   | NYAEQFF       |       |            |          |             |
| TGTGCCAGCAGC      | CAGACTGGGGGCACG | AACTATGCTGACAGTCCTTC | 6    | CD8\( \alpha \) | \( \nu\beta 11-D\beta 2-J\beta 2.1 \) | 12           |
| CASSI             | NRR         | NSPL          |       |            |          |             |
| TGTGCCAGCAGT      | AAACAGA     | AATTCGCCCTCTCTC | 4    | CD8\( \alpha \) | \( \nu\beta 06-D\beta 1-J\beta 1.6 \) | 8            |
| CASSI             | SYY         | YEQYYF        |       |            |          |             |
| TGTGCCAGCAGT      | CAG         | CTATGAACAGTACTTC | 2    | CD8\( \beta \) | \( \nu\beta 06-D\beta 1-J\beta 2.7 \) | 6            |
| CASSI             | TGE         | YSPL          |       |            |          |             |
| TGTGCCAGCAGT      | CACAGGGGG   | ATTCGCCCTCTCTC | 3    | CD8\( \beta \) | \( \nu\beta 06-D\beta 1-J\beta 1.6 \) | 9            |
| CASS              | GTGGG       | YAEQ          |       |            |          |             |
| TGTGCCAGCAGT      | GGGACAGGGGG | CTATGCTGAGCAG  | 2    | CD8\( \beta \) | \( \nu\beta 82-D\beta 1-J\beta 2.1 \) | 9            |
| CASSD             | GGGGGAA     | GTGGGCTCGCTCTC | 3    | CD8\( \beta \) | \( \nu\beta 82-D\beta 1-J\beta 2.2 \) | 8            |
| CASS              | WSGG        | QDQFY         |       |            |          |             |
| TGTGCCAGCAGC      | TGGAAGGGGG  | CAACCAACCACTTTT | 3    | CD8\( \beta \) | \( \nu\beta 10-D\beta 2-J\beta 2.5 \) | 9            |
| CASS              | TGTGV       | YEYQQYF       |       |            |          |             |
| TGTGCCAGCAGC      | ACCGGGACTGGG | CTATGACAGTACTTC | 2    | CD8\( \beta \) | \( \nu\beta 11-D\beta 2-J\beta 2.7 \) | 9            |
| CASS              | HPP         | TEVFF         |       |            |          |             |
| TGTGCCAGCAGC      | CACCC       | CACAGAAGTCCTTCTTC | 6\*  | CD8\( \beta \) | \( \nu\beta 11-D\beta ?-J\beta 1.1 \) | 6            |

D region sequence that cannot be definitively assigned to \( D\beta 1 \) or \( D\beta 2 \) is assigned by a question mark. The 3' end of the \( \nu\beta 6 \) and \( \nu\beta 11 \) 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 KNA 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⁺               | Vß06-Dß1-Jß1.6       | 3                | 7              | 2  | 3        | 8              |              |                |   |          |                |
| 2     | CD8β⁺               | Vß06-Dß1-Jß2.7       | 4                | 3              | 5  | 0        | 5              |              |                |   |          |                |
| 3     | CD8β⁺               | Vß10-Dß2-Jß2.5       | 1                | 10             | 3  | 1        | 3              |              |                |   |          |                |
| 6 (up-md-lw) | CD8β⁺         | Vß11-ßB α-Jß1.1     | 1                | 3              | 2  | 7        | 2              |              |                |   |          |                |
| 2     | CD8β⁺               | Vß11-Dß2-Jß2.7       | 3                | 10             | 4  | 1        | 5              |              |                |   |          |                |
| 3     | CD8β⁺               | Vß06-Dß1-Jß2.2       | 1                | 5              | 5  | 2        | 8              |              |                |   |          |                |
| 4     | CD8β⁻               | Vß06-Dß1-Jß1.5       | 1                | 6              | 6  | 1        | 3              |              |                |   |          |                |
| 3     | CD8β⁻               | Vß10-Dß1-Jß1.2       | 4                | 6              | 2  | 0        | 2              |              |                |   |          |                |
| 2     | CD8β⁻               | Vß10-Dß1-Jß2.5       | 5                | 5              | 1  | 2        | 2              |              |                |   |          |                |
| 6 (lw) | CD8β⁻              | Vß11-Dß2-Jß2.1     | 7                | 12             | 0  | 3        | 1              |              |                |   |          |                |

For CD8β⁺ IEL, mean CDR3 length = 8.00 nt, mean V-D length = 1.00 nt, mean D length = 2.29 nt, mean Exo 3' V⁺ = 3.43, mean Exo 5' D⁺ = 6.86, mean Exo 3' D⁻ = 1.29, mean N-D-J = 5.14.

For CD8β⁻ IEL, mean CDR3 length = 9.40 nt, mean V-D length = 1.80 nt, mean D length = 3.80 nt, mean Exo 3' V⁺ = 1.80, mean Exo 5' D⁺ = 6.80, mean Exo 3' D⁻ = 1.80, mean N-D-J = 2.60.

For CD8β⁺ and CD8β⁻ IEL, mean CDR3 length = 8.58 nt, mean V-D length = 1.33 nt, mean D length = 2.92 nt, mean Exo 3' V⁺ = 2.75, mean Exo 5' D⁺ = 6.83, mean Exo 3' D⁻ = 1.25, mean N-D-J = 4.08.

D region sequence that cannot be definitively assigned to Dß1 or Dß2 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 Vß, Dß, and Jß 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

| Vβ | Jβ | CDR3 length | Section of intestine (Percentage in the given Vβ) | CD8αα IEL | CD8αβ IEL |
|----|----|-------------|-------------------------------------------------|----------|----------|
|    |    |             | up    | md | lw | up    | md | lw |
| 6  | 1.1| 8           | --    | 12 | 19 | --    | -- | -- |
| 6  | 1.5| 8           | 42*   | 28*| 65*| --    | -- | -- |
| 6  | 2.7| 8           | 31    | 35 | 28 | 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 median 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 x 10⁶ 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 in the thymus of (C57BL/B6 x DBA/2)F1 mice (26). This did not prevent us from observing complex size patterns of CDR3 lengths in the lymph node samples. Mls-1-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 β2-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 CD8αα 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 temporarily found in the gastrointestinal canal but they do not colonize it except under abnormal circumstances (35).

The case of the CD8αβ 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 oligodonality of those IEL which are the progeny of blasts antigenically stimulated in the Peyer's patches, and the differences of these oligodonal 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 oligodonality 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 oligodonal 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 to the top of the villosities where they are shed in the gut lumen.

How could the oligodonality of the CD8αα 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 CD8αα 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 CD8αβ IEL, CD8αα 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 αEβ7 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 CD8αα and CD8αβ 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.

We thank Sylvie Darche and Michelle Malassis-Seris for their technical assistance; Françoise Selz for cell sorting; Antonio Bandeira, Richard Ferrero, and Philippe Lagrange for helpful discussions; and David Ojcius and Pablo Pereira for critical reading of the manuscript.
References

1. Bonneville, M., C.A. Janeway, K. Ito, W. Haser, I. Ishida, N. Nakanishi, and S. Tonegawa. 1988. Intestinal intraepithelial lymphocytes are distinct set of γδ T cells. Nature (Lond.). 336:479.

2. De Geus, B., M. Van den Enden, C. Coolen, L. Nagelkerken, P. Van der Heijden, and J. Rozing. 1990. Phenotype of intraepithelial lymphocytes in euthymic and athymic mice: implications for differentiation of cells bearing a CD3-associated γδ T cell receptor. Eur. J. Immunol. 20:291.

3. Goodman, T., and L. Lefrançois. 1989. Expression of the γδ T cell receptor on murine intestinal intraepithelial lymphocytes. Nature (Lond.). 335:855.

4. Guy-Grand, D., N. Cerf-Bensussan, B. Malissen, M. Malassis-Seris, C. Briottet, and P. Vassalli. 1991. Two gut intraepithelial CD8+ lymphocyte populations with different T cell receptors: a role for gut epithelium in T cell differentiation. J. Exp. Med. 173:471.

5. Takagaki, Y., A. DeCloux, M. Bonneville, and S. Tonegawa. 1989. Diversity of γδ T-cell receptors on murine intestinal intraepithelial lymphocytes. Nature (Lond.). 339:712.

6. Bandeira, A., T. Mota-Santos, S. Itchora, S. Degermann, S. Heusser, S. Tonegawa, and A. Coutinho. 1990. Localization of γδ T cells to the intestinal epithelium is independent of normal microbial colonization. J. Exp. Med. 172:229.

7. Bandeira, A., S. Itchora, M. Bonneville, O. Burlen-Defranoux, T. Mota-Santos, A. Coutinho, and S. Tonegawa. 1991. Extra-thymic origin of intestinal intraepithelial lymphocytes bearing T-cell antigen receptor γδ. Proc. Natl. Acad. Sci. USA. 88:43.

8. Poussier, P., P. Edouard, C. Lee, M. Binnie, and M. Julius. 1992. Thymus-independent development and negative selection of T cell receptor α/β in the intestinal epithelium: evidence for distinct circulation patterns of gut- and thymus-derived T lymphocytes. J. Exp. Med. 167:387.

9. Guy-Grand, D., C. Griscelli, and P. Vassalli. 1978. The mouse gut T lymphocyte, a novel type of T cell. Nature, origin, and traffic in mice in normal and graft-versus-host conditions. J. Exp. Med. 148:1661.

10. Guy-Grand, D., and P. Vassalli. 1986. Gut injury in mouse graft-versus-host reaction. Study of its occurrence and mechanisms. J. Clin. Invest. 77:1584.

11. Halstead, T.E., and J.G. Hall. 1972. The homing of lymphoborne immunoblasts to the small gut of neonatal rats. Transplantation (Baltimore). 14:339.

12. Sprent, J. 1976. Fate of H2-activated T lymphocytes in syngeneic hosts. Cell. Immunol. 21:278.

13. Mosley, R.L., and J.R. Klein. 1992. Peripheral engraftment of fetal intestine into athymic mice sponsors T cell development: direct evidence for thymopoietic function of murine small intestine. J. Exp. Med. 176:1365.

14. Poussier, P., H.S. Teh, and M. Julius. 1993. Thymus-independent positive and negative selection of T cells expressing a major histocompatibility complex class I restricted transgenic T cell receptor α/β in the intestinal epithelium. J. Exp. Med. 178:1947.

15. Rocha, B., P. Vassalli, and D. Guy-Grand. 1994. Thymic and extrathymic origins of gut intraepithelial lymphocyte populations in mice. J. Exp. Med. 180:681.

16. Guy-Grand, D., B. Rocha, P. Minka, M. Malassis-Seris, F. Selz, B. Malissen, and P. Vassalli. 1994. Different use of the T cell receptor transducing modules in two populations of gut intraepithelial lymphocytes are related to distinct pathways of T cell differentiation. J. Exp. Med. 180:673.

17. Balk, S.P., E.C. Ebert, R.L. Blumenthal, F.V. McDermott, K.W. Wucherpfenning, S.B. Landau, and R.S. Blumberg. 1991. Oligoclonal expansion and CD1 recognition by intestinal intraepithelial lymphocytes. Science (Wash. DC). 253:1411.

18. Blumberg, R.S., C.E. Yockey, G.G. Gross, E.C. Ebert, and S.P. Balk. 1993. Human intestinal intraepithelial lymphocytes are derived from a limited number of T cell clones that utilize multiple Vβ T cell receptor genes. J. Immunol. 150:5144.

19. Van Kerkhove, C., G.J. Russel, K. Deutsch, K. Reich, A.K. Bhan, H. DerSimonian, and M.B. Brenner. 1992. Oligoclonality of human intestinal intraepithelial T cells. J. Exp. Med. 175:57.

20. Panneietier, C., M. Crochet, S. Darche, A. Casrouge, M. Zöller, and P. Kouiris. 1993. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor β chains vary as a function of the recombined germ-line segments. Proc. Natl. Acad. Sci. USA. 90:4319.

21. Kabat, E.A., T.T. Wu, H.M. Perry, K.S. Gottesman, and C. Foeller. 1991. Sequences of Proteins of Immunological Interest. U.S. Department of Health and Human Services, Fifth Edition, NIH Publication.

22. Casanova, J.L. 1990. Optimal conditions for directly sequencing double-stranded PCR products with sequenase. Nucleic Acids Res. 18:4028.

23. Crochet, M., C. Panpetier, A. Regnault, S. Darche, C. Leclerc, and P. Kouiris. 1992. Molecular detection and in vivo analysis of the specific T cell response to a protein antigen. Eur. J. Immunol. 22:2639.

24. Tononari, K., S. Fairchild, and O. Rosenwasser. 1993. Influence of viral superantigens on Vβ and Vα-specific positive and negative selection. Immunol. Rev. 131:131.

25. Rocha, B., P. Vassalli, and D. Guy-Grand. 1991. The Vβ repertoire of mouse gut homodimeric αCD8 intraepithelial T cell receptor α/βγδ lymphocytes reveals a major extrathymic pathway of T cell differentiation. J. Exp. Med. 173:483.

26. McDonald, H.R., R. Schneider, R.K. Lees, R.C. Howe, H. Acha-Orbea, H. Festenstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ usage predicts reactivity and tolerance to Mls-encoded antigens. Nature (Lond.). 332:40.

27. Candias, S., C. Waltzinger, C. Benoist, and D. Mathis. 1991. The Vγ/δ T cell repertoire: skewed Jδ usage after thymic selection; dissimilar CDR3s in CD4+ versus CD8+ cells. J. Exp. Med. 174:989.

28. Asarnow, D.M., T. Goodman, L. Lefrançois, and J.P. Allison. 1989. Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells. Nature (Lond.). 341:60.
29. Whetsell, M., R.L. Mosley, L. Whetsell, F.V. Schaefer, K.S. Miller, and J.R. Klein. 1991. Rearrangement and junctional-site sequence analyses of T-cell receptor gamma genes in intestinal intraepithelial lymphocytes from murine athymic chimeras. *Mol. Cell. Biol.* 11:5902.

30. Landau, N.R., D.G. Schatz, M. Rose, and D. Baltimore. 1987. Increased frequency of N-region insertion in a murine pre-B-cell line infected with a terminal deoxynucleotidyl transferase retroviral expression vector. *Mol. Cell. Biol.* 7:3237.

31. Correa, I., M. Bik, N.-S. Liao, M. Zijlstra, R. Jaenisch, and D. Raulet. 1992. Most γδ T cells develop normally in β2-microglobulin-deficient mice. *Proc. Natl. Acad. Sci. USA.* 89:653.

32. Schleussner, C., and K. Ceredig. 1993. Analysis of intraepithelial lymphocytes from major histocompatibility complex (MHC)-deficient mice: no evidence for a role of MHC class II antigens in the positive selection of Vβ8* γδ T cells. *Eur. J. Immunol.* 23:1615.

33. Rocha, B., H.V. Boehmer, and D. Guy-Grand. 1992. Selection of intraepithelial lymphocytes with CD8α/α receptors by self-antigen in the murine gut. *Proc. Natl. Acad. Sci. USA.* 89:5336.

34. Savage, D.C. 1977. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.* 31:107.

35. Savage, D.C. 1977. Interactions between the host and its microbes. In *Microbial Ecology of the Gut.* R.T.J. Clarke, and T. Bauchop, editors. Academic Press Inc., London. 410.

36. Lin, T., G. Matsuzaki, H. Kenai, and K. Nomoto. 1993. Thymus influence the development of extraviscerally derived intestinal intrapithelial lymphocytes. *Eur. J. Immunol.* 23:1615.

37. Guy-Grand, D., C.V. Broecke, C. Briottet, M. Malassis-Seris, F. Selz, and P. Vassalli. 1992. Different expression of the recombination activity gene RAG-1 in various populations of thymocytes, peripheral T cells and gut thymus-independent intraepithelial lymphocytes suggests two pathways of T cell receptor rearrangement. *Eur. J. Immunol.* 23:1615.

38. Cerf-Bensussan, N., A. Jarry, N. Brousse, B. Lisowska-Grosjean, D. Guy-Grand, and C. Griscelli. 1987. A monoclonal antibody (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *Eur. J. Immunol.* 17:1279.

39.  

1358 Oligoclonal Repertoire of the CD8αα and the CD8αβ TCR-α/β Murine IELs