T Cell Receptor Dynamism of Mucosal and Systemic CD4\(^+\) T Cells in the Course of an Immune Response to *Escherichia coli* Heat-Labile Enterotoxin

Jin-Kyung Kim, Ichiro Takahashi, Yoshiko Okuda, Mitsuo Itakura, Jerry R. McGhee, and Hiroshi Kiyono

The changes in T cell receptor (TCR) V\(\beta\) expression, use, and clonality in mice orally challenged with *Escherichia coli* heat-labile enterotoxin (LT) were assessed. Use of the TCR V\(\beta\) family and clonality were significantly changed at the single-cell level. In Peyer’s patches of treated mice, use of TCR V\(\beta\)6, V\(\beta\)8, and V\(\beta\)14 increased in CD4\(^+\)CD4\(^+\) T cells, compared with use in nontreated mice. On the other hand, use of TCR V\(\beta\)1 and V\(\beta\)8 was enhanced in splenic CD4\(^+\)CD4\(^+\) T cells. Intraepithelial lymphocytes isolated from LT-challenged mice showed expanded clonality (e.g., V\(\beta\)1, V\(\beta\)2, V\(\beta\)9, and V\(\beta\)18) and altered TCR V\(\beta\) use (e.g., V\(\beta\)15, V\(\beta\)16, and V\(\beta\)17). These findings reveal that oral administration of LT has distinct effects on mucosal versus systemic CD4\(^+\) T cells for induction of CD4\(^+\) T cells with selected V\(\beta\) use. This most likely reflects the function of LT as a mucosal modulator.

*Escherichia coli* heat-labile enterotoxin (LT), which induces a diarrheogenic response in humans, is composed of 2 non–covalently bound subunits, A and B (LT-A and LT-B). The biologic activity of the toxin is due to the ability of LT-A to covalently bind subunits, A and B (LT-A and LT-B). The a diarrheogenic response in humans, is composed of 2 non–

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antigen recognition during infection, it is important to determine TCR Vβ use and clonotype of antigen-specific T cells.

Since LT binds to mucosal cells via ganglioside receptors, we hypothesized that this toxin would induce different T cell repertoires in the gastrointestinal tract than in systemic lymphoid tissues. To verify this possibility, we determined the TCR Vβ use and clonotype of CD4+ T cells isolated from mucosal and systemic lymphoid tissues of mice orally challenged with LT.

Materials and Methods

Mice. Female BALB/c mice (H-2b; 6 weeks old; Charles River Japan) were used in this study. All mice were maintained in the Experimental Animal Facility at the Research Institute for Microbial Diseases, Osaka University.

Treatment of mice. For oral challenge, LT (Sigma Chemical) was resuspended in PBS (250 μg/mL). Mice (3–5 per group) were each given 50 μg of LT intragastrically on days 0, 7, and 14. Fecal and serum samples were collected at weekly intervals and were monitored for the induction of LT-specific IgG and IgA antibodies, as described elsewhere [24].

Isolation of lymphocytes from mucosal tissues and spleens. Mice were killed at weekly intervals after oral administration of LT. Spleens were aseptically removed, and single-cell suspensions were prepared by a standard mechanical disruption procedure, as described elsewhere [25]. Single-cell suspensions of Peyer’s patches were prepared by an enzymatic dissociation method using collagenase (Nitta gelatin), as described elsewhere [26]. Intraepithelial lymphocytes (IELs) were isolated by a standard mechanical dissociation method, as described elsewhere [27]. In brief, Peyer’s patches, fat, and mesentery were removed from the small intestine. The gut tissue was resuspended in PBS (250 mM NaCl, 10 mM NaHCO3, 1 mM MgCl2, 2.5% Nonidet P-40 (NP-40; pH 7.5) and IELs, was collected, and the incubation was repeated twice more with RPMI 1640 alone. Cells were added to the top of a 40% Percoll (Pharmacia Biotech) gradient and were centrifuged at 600 g for 20 min. Cells at the 40% and 75% interface were collected and were washed in RPMI 1640 containing 10% fetal calf serum. A separation system (MACS; Miltenyi Biotec) was used to isolate purified CD4+ T cells. In brief, the CD3+ T cell fraction was obtained by negative panning on petri plates coated with F(ab)2 goat anti-mouse IgG (Jackson Immunoresearch Laboratories). Three rounds of panning were done, to remove adherent macrophages and B cells. The T cell population was further incubated with anti-CD4-conjugated microbeads (L3T4; Miltenyi Biotec) at 4°C for 15 min and then was passed through the magnetic column (Miltenyi Biotec). The purity of these T cell suspensions was >97% for the CD4+ T cells, as shown by flow cytometry.

Flow cytometric analysis. To characterize the T cell subsets in lymphocytes isolated from spleens, Peyer’s patches, and IELs, we stained cells with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (Pharmingen), unless otherwise indicated, anti-TCR β (H57–597), anti-CD4 (L3T4), TCR Vβ2 (B20.6), Vβ3 (KJ25), Vβ4 (KT4), Vβ5.1 (MR9–4), Vβ6 (RR4–7), Vβ7 (TR3–10), Vβ8.1, Vβ8.2 (MR5–2), Vβ8.3 (1B3.3), Vβ9 (MR10–2), Vβ10 (B21.5), Vβ11 (RR3–15), Vβ12 (MR11–1), Vβ13 (MR12–3), Vβ14 (MR 14–2), and phycoerythrin (PE)–conjugated anti–TCR β (GL3), CD4 (L3T4), and CD44 (Pgp-1). Flow cytometric analysis (FACScan; Becton Dickinson) was done and was analyzed with CellQuest software (Becton Dickinson). Lymphocytes were gated by size and density, on the basis of forward and side scatter.

Analysis by reverse transcription (RT)–polymerase chain reaction (PCR) and PCR single-stranded conformational polymorphism (SSCP). RT-PCR analysis was done to detect the TCR Vβ-specific mRNA expression, as described elsewhere [21, 28]. For the isolation of RNA, TRizol reagent (Life Technologies) was used, according to the manufacturer’s protocol. cDNA was synthesized from 50 ng of extracted RNA by using the GeneAmp RNA PCR Core kit (Perkin-Elmer). PCR amplification (GeneAmp PCR system 9600; Perkin-Elmer) was done in sequential cycles at 94°C for 1 min and 60°C for 1 min, with a 72°C extension step for 7 min after the last cycle. All samples were amplified for 35 cycles. The sequences of the primers used are shown in table 1. For fluorescence-based PCR-SSCP analysis [28], the 6-carboxyfluorescein–labeled Cβ primer was purchased from Invitrogen. The PCR products were diluted 20 times with a loading buffer consisting of formamide and EDTA. The DNA samples were heated at 95°C for 5 min and were cooled immediately on ice. This solution (5 μL) was applied to a non-denaturating 8% polyacrylamide gel set to the Applied Biosystems model 373A and GeneScan 672 computer software (Perkin-Elmer/Applied Biosystems). Red-colored double-strand GeneScan 500 ROX (Applied Biosystems) was used as an internal DNA size marker.

Single-cell RT-PCR. To isolate CD4+CD44+ T cells from spleens, Peyer’s patches, and IELs, mononuclear cells were incubated simultaneously with FITC-conjugated anti-CD4 (L3T4; Pharmingen) and PE-conjugated anti-CD44 (Pgp-1; Pharmingen), and a positive selection was performed by use of FACS (Becton Dickinson). Single cells were sorted into the wells of 96-well microplates (Becton Dickinson) containing 150 mM KCl, 100 mM Tris-HCl (pH 8.5), 7.5 mM MgCl2, 2.5% Nonidet P-40 (NP-40; pH 7.5) and 0.1% Tween-20. cDNA and the primers were amplification conditions (94°C for 1 min, annealing 1 min, and 72°C extension step for 2 min) of the primers used are shown in table 1. For fluorescence-based PCR-SSCP analysis [28], the 6-carboxyfluorescein–labeled Cβ primer was purchased from Invitrogen. The DNA samples were heated at 95°C for 5 min and were cooled immediately on ice. This solution (5 μL) was applied to a non-denaturating 8% polyacrylamide gel set to the Applied Biosystems model 373A and GeneScan 672 computer software (Perkin-Elmer/Applied Biosystems). Red-colored double-strand GeneScan 500 ROX (Applied Biosystems) was used as an internal DNA size marker.

Table 1. Sequence of primers used for reverse transcription–polymerase chain reaction.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vβ1</td>
<td>ACAGTTGATTCGAATGAGACGG</td>
</tr>
<tr>
<td>Vβ2</td>
<td>AGTCTGGGGGACAAAGAGGTCAAA</td>
</tr>
<tr>
<td>Vβ3</td>
<td>CTTTCAGAATCAAGAAGTTCCTC</td>
</tr>
<tr>
<td>Vβ4</td>
<td>TTATGGAACACTGAGTCTC</td>
</tr>
<tr>
<td>Vβ5</td>
<td>GGAGAGAGGATTTTTTCTGCTG</td>
</tr>
<tr>
<td>Vβ6</td>
<td>AAGGCGAGTCTATGAGCTAT</td>
</tr>
<tr>
<td>Vβ7</td>
<td>TAGATTCAGGGAGGAGCACTCCC</td>
</tr>
<tr>
<td>Vβ8</td>
<td>ACAGGGAGAAGCTGAGGAGTTGAC</td>
</tr>
<tr>
<td>Vβ9</td>
<td>GATTTTGAGAAGGAGGATCAGATCT</td>
</tr>
<tr>
<td>Vβ10</td>
<td>GCTTTCCACCTGTCCTGCTAG</td>
</tr>
<tr>
<td>Vβ11</td>
<td>TTGATGAGATGCTGAGGAGTCC</td>
</tr>
<tr>
<td>Vβ12</td>
<td>TTGATGAGATGCTGAGGAGTCC</td>
</tr>
<tr>
<td>Vβ13</td>
<td>TGGATGAGATGCTGAGGAGTCC</td>
</tr>
<tr>
<td>Vβ14</td>
<td>ATTACTTGTTGGCAGCTGAGT</td>
</tr>
<tr>
<td>Vβ15</td>
<td>GCACCCCTAGCTATGAC</td>
</tr>
<tr>
<td>Vβ16</td>
<td>GGTTAAAGCTGAGGAGGAGTCT</td>
</tr>
<tr>
<td>Vβ17</td>
<td>GGAGGACCAAGCTGGTGACAGAAGA</td>
</tr>
<tr>
<td>Vβ18</td>
<td>GGACCCAAGGCTGAATCCTCAGG</td>
</tr>
<tr>
<td>Vβ19</td>
<td>CCTACATGAAAGGAGGGAGT</td>
</tr>
<tr>
<td>Cβ</td>
<td>GTGGAAGTCATTTTCTCAGATC</td>
</tr>
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</table>
The RT reaction was carried out by incubating the plates at 42°C for 60 min after adding 2 U of RT (Superscript II; Life Technologies). To amplify cDNA, random PCR amplification was performed. Ten microliters of RT products was added into 40 μL of random PCR mixture containing 100 μM KCl, 2.5 μM MgCl2, 3 μg of random hexamer (Life Technologies), and 2.5 μL of Taq DNA polymerase (Pharmacia). The random PCR amplification proceeded through the following scheme: 30 cycles of 20 s at 94°C, 10 s at each degree from 37°C to 65°C, and 3 min at 68°C. To select the β-actin–positive mixture, the PCR reaction was started by adding 1 μM β-actin primer and 1.25 U of Taq Gold DNA polymerase to 6 μL of random PCR product. One hundred β-actin–positive samples from each group were selected for the characterization of TCR Vβ expression.

Statistical analysis. The results are expressed as the mean ± 1 SD, and statistical significance (P < .05) was determined by Student’s t test.

Results

LT-induced, CD44+–type CD4+ T cell responses to oral challenge in mucosa-associated and systemic lymphoid tissues. We first analyzed the effect of LT as an immunogen by investigating the levels of antigen-specific IgG and IgA antibodies in serum and fecal extracts of mice orally challenged with LT. Levels of LT-specific serum IgG and fecal IgA were elevated on days 14 and 28 after the first administration, which confirmed our earlier results (data not shown) [13, 24]. Furthermore, analysis of antigen-specific IgG subclass responses revealed that serum IgG subclass IgG2a was induced in mice given oral LT, in addition to IgG1 antibodies. This observation supports our earlier reports that oral administration of LT enhances the mucosal (e.g., IgA) and systemic (e.g., IgG1 and IgG2a) antibody responses via the coinduction of Th1- and Th2-type cytokine-producing CD4+ T cells [13, 24].

To further elucidate the effects of orally administered LT on mucosal and systemic T cells, our initial experiment was aimed at determining the influence of LT on the generation of memory-activated (CD44+ CD69+) T cells. To this end, the lymphocytes isolated from spleens, Peyer’s patches, and IELs of mice orally challenged with LT were double-stained with FITC-conjugated anti-CD4 and PE-conjugated anti-CD44. An increase in the frequency of CD4+ CD44+ T cells was seen in both systemic and mucosal sites (namely, spleens, Peyer’s patches, and IELs) on day 28 after oral challenge (figure 1). The frequency of CD4+ CD44+ T cells was not changed in PBS-fed control mice on day 28; thus, the increased frequency in LT-treated mice indicates that LT can induce CD44+–type CD4+ T cells in both mucosa-associated and systemic lymphoid tissues.

Increases in the proportion of αβ but not γδ T cells in LT-challenged mice. To further understand the mechanisms underlying the effects of LT on T cells, we next investigated the numbers of splenic, Peyer’s patches, and intraepithelial αβ T cells in mice orally challenged with LT. It was interesting to note that cell numbers were increased in spleens ([4.72 ± 3.00] × 10^7) and Peyer’s patches ([9.97 ± 1.47] × 10^7) of LT-fed mice on day 28, compared with those on day 0 ([3.65 ± 2.82] × 10^7) and [8.14 ± 0.08] × 10^7, respectively. In the case of IELs, the numbers of αβ T cells in controls were slightly elevated ([1.50 ± 0.10] × 10^7) on day 28, compared with those in pretreated mice ([1.40 ± 0.49] × 10^7). In contrast, the numbers of γδ T cells were decreased (from [0.70 ± 0.02] × 10^7 to [0.28 ± 0.01] × 10^7) by oral administration of LT. These findings were more obvious when the proportions of αβ and γδ T cell subsets in mucosa-associated (Peyer’s patches and IELs) and systemic lymphoid (spleen) tissues were examined. Slight increases in αβ T cell counts were noted in spleens and Peyer’s patches of treated, compared with nontreated, mice (figure 2). Furthermore, the frequency of αβ T cells in IELs from mice orally challenged with LT dramatically increased (figure 2). On the other hand, the frequency of γδ T cells was lower after oral LT. In addition, when we administered LT subcutaneously, the frequency of αβ T cells was expanded in spleens but not in mucosal sites, such as Peyer’s patches and IELs (data not shown).

These findings indicated that systemic challenge with LT expanded the αβ T cells only in the systemic compartment, whereas oral challenge influenced expansion in both mucosal and systemic sites. These results suggest that orally administered LT enhanced the proportion of αβ T cells but not γδ T cells, in both mucosal and systemic lymphoid tissues. Furthermore, it is important to note that analysis of LT-B–fed mice did not change the proportion of αβ and γδ T cell subsets among the spleens, Peyer’s patches, and IELs.

Analysis of TCR Vβ expression, use, and clonality by orally administered LT. To further investigate the expanded numbers of αβ T cells in both mucosa-associated and systemic lymphoid tissues of mice orally challenged with LT, our next experiment was aimed at characterizing the expression of different TCR Vβ families by double staining with FITC-conjugated anti-Vβ–specific monoclonal antibodies and PE-conjugated anti-CD4. Oral administration of LT did not change the pattern of TCR Vβ expression in either systemic or mucosa-associated lymphoid tissues, with the exception that TCR Vβ6 T cells were increased in CD4+ T cells of IELs on day 28 (figure 3). A similar pattern was also noted when CD4+ T cells from these tissues were isolated on day 7 or 14 and were assessed for TCR Vβ expression (data not shown).

To determine whether TCR Vβ use and clonotype of CD4+ T cells were altered by oral administration of LT, RT-PCR amplification and PCR-SSCP analysis were performed on CD4+ T cells isolated from spleens, Peyer’s patches, and IELs (figure 4). The PCR-SSCP analysis used in this study has been shown to be sensitive, since a corresponding band can be detected when a T cell clone exists at a frequency of <1 in 1000 [30]. Amplified CDR3-specific DNA originating from 1 T cell
Figure 1. Frequency (in counts) of CD44<sup>+</sup> cells in CD4<sup>+</sup> T cell subsets isolated from spleens, Peyer’s patches, and intraepithelial lymphocytes (IELs) of control mice and mice treated with *Escherichia coli* heat-labile enterotoxin (LT). A, Representative flow cytometry profile of CD4<sup>+</sup>CD44<sup>+</sup> T cells isolated from pretreated mice (day 0) and from PBS-fed (day 28–PBS) and LT-fed (day 28–LT) mice 28 days after the first oral dose. To determine the expression of CD44, cells were stained with fluorescein isothiocyanate–conjugated anti-CD4 (L3T4) in the presence of anti-CD44–specific monoclonal antibody. Shown are histogram analyses for surface expression of CD44 gated on CD4<sup>+</sup>T cells. Dotted (lighter) lines indicate isotype-matched (negative) controls. Nos. indicate the percentage of the gated area. PE, phycoerythrin. B, Mean percentages of CD44<sup>+</sup>T cells in the CD4<sup>+</sup>T cell subsets. Bars indicate mean of 3 independent experiments for proportions of CD44<sup>+</sup> cells in the CD4<sup>+</sup>T cell subset. *P < .001 by Student’s t test, compared with pretreated mice (day 0).

clone can be visualized as a single band in the SSCP gel, reflecting its uniquely folded conformation, whereas amplified DNA from a diverse T cell population exhibits a smear pattern composed of innumerable faint bands.

Oral delivery of LT induced the expansion of TCR V<sub>β</sub> use and the clonality of CD4<sup>+</sup> T cells in mucosal lymphoid tissues. The expansion of TCR V<sub>β</sub>6, V<sub>β</sub>8, V<sub>β</sub>13, V<sub>β</sub>14, and V<sub>β</sub>15 families was noted in Peyer’s patches of treated, compared with control, mice (figure 4). IELs also exhibited a distinct alteration in both the TCR V<sub>β</sub> use and clonotype in mice orally challenged with LT. The use of TCR V<sub>β</sub>15, V<sub>β</sub>16, and V<sub>β</sub>17 was expanded in LT-administered mice, whereas no bands were noted in control mice. In addition, TCR V<sub>β</sub>1, V<sub>β</sub>2, V<sub>β</sub>9, and V<sub>β</sub>18 genes were clonally expanded in IELs isolated from mice orally challenged with LT. However, these obvious changes were not seen in splenic CD4<sup>+</sup> T cells isolated from the same mice. Taken
Figure 2. Proportions of \( \alpha \beta \) and \( \gamma \delta \) T cell subsets isolated from spleens, Peyer’s patches, and intraepithelial lymphocytes (IELs) of mice orally challenged with Escherichia coli heat-labile enterotoxin (LT). A, Representative flow cytometry profiles of spleen, Peyer’s patches, and IELs in pretreated mice (day 0) and in mice 28 days after the first oral administration of LT (day 28). Cells were isolated and were double-stained with fluorescein isothiocyanate–conjugated T cell receptor (TCR)-\( \beta \) and phycoerythrin-conjugated TCR-\( \delta \) monoclonal antibodies. Percentages of cells for a given phenotype are shown. B, Mean percentages of \( \alpha \beta \) and \( \gamma \delta \) T cell subsets. Bars indicate mean percentage ± SD of 3 independent experiments for proportions of \( \alpha \beta \) (left panel) and \( \gamma \delta \) (right panel) T cells. * \( P < .05 \) by Student’s \( t \) test, compared with pretreated mice (day 0).

Together, these data suggest that the immunomodulatory activity of LT on mucosal T cells might be different from that seen on T cells in the periphery.

**Single-cell analysis of TCR \( V_{\beta} \) use and clonotypic analysis.** To further confirm the above results and to analyze at the single-cell level the use and clonotype of CD4\(^+\) T cells in Peyer’s patches, IELs, and spleens of mice orally challenged with LT, a single-cell RT-PCR procedure was adopted for the subsequent experiment. To this end, CD4\(^+\)CD44\(^+\) T cells from Peyer’s patches, IELs, and spleens of mice orally challenged with LT were sorted into single cells by flow cytometry. We did not observe any dramatic change in \( V_{\beta} \) use when splenic CD4\(^+\) T cells from mice orally administered LT were characterized by SSCP (figure 4); however, a restricted change was noted, in
Values are mean of 3 independent experiments. *percentage of CD4 T cells bearing each TCR Vβ subset.

**Figure 3.** Flow cytometric analysis of T cell receptor (TCR) Vβ expression by CD4+ T cells from the spleen, Peyer’s patch, and intraepithelial lymphocytes (IELs) in pretreated mice (day 0) and in mice 28 days after the first oral administration of *Escherichia coli* heat-labile enterotoxin (day 28). Cells were double-stained with fluorescein isothiocyanate–conjugated TCR Vβ and phycoerythrin-conjugated CD4. The percentage of CD4+ T cells bearing each TCR Vβ was calculated as 100 × (%Vβ3 + Vβ4 + Vβ5 + Vβ6 + Vβ7) × (%Vβ2 + Vβ3 + Vβ4 + Vβ5.1 + Vβ6 + Vβ7 + Vβ8.1 + Vβ8.2 + Vβ8.3 + Vβ9 + Vβ10 + Vβ11 + Vβ12 + Vβ13 + Vβ14)]. Values are mean percentage ± SD of 3 independent experiments. *P < .05 by Student’s t test, compared with pretreated mice (day 0).

which highly purified splenic CD4+CD44+ T cells expressing TCR Vβ1 and Vβ8 gene products increased following oral administration of LT (figure 5). In the case of Peyer’s patches, the use of TCR Vβ6, Vβ8, and Vβ14 genes increased after LT challenge (figure 5); however, there was no significant alteration of IELs by oral administration of LT at the single-cell level.

TCR Vβ clonotypes of these CD4+CD44+ T cells in Peyer’s patches, IELs, and spleens from mice orally administered LT were also examined by the PCR-SSCP method. IELs and splenic T cells from challenged mice showed no remarkable changes in clonotype, compared with those in control mice. On the other hand, skewing of the clonality was noted in the TCR Vβ14 and Vβ15 subsets of Peyer’s patch T cells after oral challenge of LT (data not shown).

**Discussion**

LT and LT-B have significant immunoregulatory potential, not only as a means of preventing the induction of oral tolerance, but also as mucosal adjuvants for orally administered antigens [1–6]. Thus, these molecules can be viewed as mucosal immunomodulators for the induction and regulation of antigen-specific immune responses to an orally administered antigen. Our earlier work demonstrated that orally administered LT induces both Th1- and Th2-type CD4+ T cell responses, which can provide 2 layers of antigen-specific immune responses in mucosal (e.g., S-IgA) and systemic (e.g., IgG1 and IgG2a) compartments [13, 24]. Even though there is some evidence to explain the immunomodulatory function of LT on T cells, the molecular mechanism is still unknown. Thus, characterization of the perturbation of the TCR Vβ repertoire of CD4+ T lymphocytes is important for understanding the underlying mechanism of those immunologic influences delivered by mucosally administered LT. This study was aimed at determining the effect of oral LT on mucosal and systemic CD4+ T cells in terms of their TCR Vβ use and clonality.

Of interest, the outcome of T cell responses was significantly different between the mucosal (Peyer’s patches and IELs) and systemic (spleen) lymphoid tissues after oral administration of LT. In this regard, oral delivery of LT resulted in an increase in αβ but not γδ T cells in both systemic and mucosal lymphoid tissues. However, the increased patterns among these different tissues were distinguishable when the proportion of αβ T cells in IELs was significantly increased by oral feeding of LT, whereas only slight increases were noted in Peyer’s patches and spleens. Analysis of TCR Vβ use and clonality also showed significant differences between systemic and mucosal lymphoid tissues. There were no changes in TCR Vβ use or clonotype of splenic CD4+ T cells from mice given oral LT; however, the mucosal lymphoid tissues, especially IELs, showed altered TCR Vβ use and clonality after oral challenge with LT. These findings suggest that orally administered LT affects TCR Vβ use and clonotype of mucosal T cells and, to a lesser extent, systemic T cells.

Several possibilities could account for the differences in the effect of LT on T cells in mucosal and systemic lymphoid tissues. The first possibility is that T cell subsets in the spleen, Peyer’s patches, and intestinal epithelium (e.g., IELs) differ in origin and thus in Vβ receptor composition [31–33]. The major T cell subsets in the spleen and Peyer’s patches are αβ T cells that originate in the thymus. In contrast, intestinal epithelium (e.g., IELs) contains extrathymically developed T cells in addition to those of thymic origin [32, 33]. Murine intestinal IELs consist of 2 main populations of αβ and γδ T cells [34]. In general, the αβ T cell population is considered to be mainly thymus derived, except for the CD8αα fraction, whereas γδ T cells are...
Figure 4. Fluorescence-based polymerase chain reaction–single-stranded conformational polymorphism analysis of T cell receptor (TCR) Vβ repertoire use and clonality of CD4+ T cells isolated from the spleen, Peyer’s patches, and intraepithelial lymphocytes (IELs) of pretreated mice (day 0) and mice 28 days after the first oral challenge with *Escherichia coli* heat-labile enterotoxin (day 28). TCR Vβ subsets with altered use or expression are indicated by arrows.
Figure 5. Single-cell reverse transcription–polymerase chain reaction analysis of T cell receptor (TCR) Vβ repertoire use in CD4+ CD44+ T cells isolated from the spleen, Peyer’s patches, and intraepithelial lymphocytes (IELs) of pretreated mice (day 0) and in mice 28 days after the first oral administration of *Escherichia coli* heat-labile enterotoxin (day 28). The percentage of T cells bearing each TCR Vβ subset was calculated as \( \% \) of TCR Vβx, on the basis of the number of actin–positive cells. Values are mean of 3 independent experiments. * by Student’s t test, compared with pretreated mice \( P < .05 \) (day 0).

thought to be thymus independent. It has been suggested recently that cryptopatches may provide the immunologic nest for extrathymically developed intestinal intraepithelial T cells [35]. Our results suggest that LT affects both thymically and extrathymically derived αβ T cells but not γδ T cells. Alternatively, orally administered LT may induce apoptosis in extrathymically developed γδ T cells in IELs, which would lead to a reduction of T cells expressing γδ TCR. In support of this possibility, it was shown that the number of intraepithelial T cells decreased in mice orally administered cholera toxin [36].

The unique environment of intestinal epithelium may explain the distinct effects of LT on mucosal versus systemic T cells. IELs are located at the basolateral surfaces of intestinal epithelial cells (IECs), which are continuously exposed to numerous environmental antigens. In this regard, IELs form a “mucosal intranet” with IECs to cooperatively respond to external stimuli provided by intestinal luminal antigens. Our earlier study demonstrated that the cytokine cross-talk between interleukin-7 and interleukin-7 receptor is an important communication element for the mucosal intranet formed by IECs and IELs [37]. Furthermore, an invasion of enteropathogenic bacteria can interfere the cytokine and its receptor-signaling pathway, which may result in a disturbance of the mucosal intranet [38]. It is interesting to further postulate that antigenic epitope(s) of LT presented by epithelial cells to mucosal T cells could be totally different from those presented by classical antigen-presenting cells, such as macrophages, dendritic cells, and B cells. Inasmuch as IELs are thought to provide the first line of defense against microbial pathogens, their ability to respond to orally encountered LT may be different from that of systemic T cells.

There is some evidence that virus infections selectively induce monoclonal or oligoclonal expansion of certain TCR Vβ populations. For example, there was a marked increase in the TCR Vβ7–, Vβ12–, Vβ14–, and Vβ17–bearing IEL subpopulation following reovirus infection in vivo [39]. In addition, this expanded TCR Vβ population showed prominent cytotoxic T cell activity. These findings suggest that subsets and clonotypes of TCR Vβ families expanded by external stimuli may be key mediators in reovirus infection [40]. Although we still must address the biologic significance of the expanded subsets, these TCR Vβ T cells may play an important role for the immunogenicity and adjuvanticity of LT in mucosal lymphoid tissues.

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