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# Regional Variations in the Distributions of Small Intestinal Intraepithelial Lymphocytes (IELs) in BALB/c +/+, *nu*/+, and *nu*/*nu* Mice

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**Regional variations in intraepithelial lymphocytes (IELs) in the small intestine were examined in BALB/c +/+, *nu*/+, and *nu*/*nu* mice. The small intestine was obtained from 11- to 12-week-old mice and divided equally into three (proximal, middle, and distal) parts. The IELs were isolated from each part of the intestine, and the total numbers of IELs in *nu*/+ and *nu*/*nu* mice were about a fifth of those in +/+ mice. Regional variations in the distribution of the IEL αβ, but not the γδ T-cell subset were found by use of flow cytometry in +/+ and *nu*/+ mice. On the other hand, such differences were not found in *nu*/*nu* mice, suggesting that thymus-independent development of T cells is not different among regions. Different local expansion of thymus-dependent αβ T cells may cause the regional variations seen in the distribution of αβ T cell IELs in +/+ and *nu*/+ mice.**

It is widely believed that a unique immune system exists in the mucosal tissues that differs markedly from the systemic immune system (1). Intraepithelial lymphocytes (IELs) are one of the components of the mucosal immune system. Many studies have been done on IELs isolated from the entire small intestine, and results of those studies have clarified that IELs contain unusual populations of T cells on the basis of expression of surface molecules (2), cytotoxic activity (2), and cytokine production (3).

The small intestine consists of three histologically evident parts (i.e., duodenum, jejunum and ileum), although their macroscopic boundaries are not clear (4). It was reported that these parts have regional variations in enzyme activities (5), intestinal flora (6), expression of MHC class-II molecules on the enterocytes (7), and number of IgA-producing cells in the lamina propria (8), as well as variations in histologic features (4).

Recently, we reported regional variations in the number and subsets of IELs distributed in the small intestine of BALB/c mice (9). Specifically, we observed that αβ T cells comprised the largest portion of the T-cell population in the distal part of the small intestine, compared with the proximal and middle parts. Within the αβ T-cell population, the $CD4^+$, $CD8^-$ T-cell and $CD4^+$ $CD8\alpha\alpha^+$ double-positive (DP) T-cell subsets are higher in the distal part, compared with the proximal and middle parts. In contrast to the αβ T-cell subset, regional variation in the small intestine was not observed within the γδ T-cell subset. In addition, we reported that these regional variations of IELs also were apparent in three inbred strains of mice as well as in male and female mice (10). These results indicate that regional variations in the small intestinal IELs are common to different mouse strains.

The purpose of the study reported here was to elucidate the influence of the thymus on regional distribution of IELs in the mouse small intestine by comparing euthymic with athymic mice. We report regional variation of thymus-dependent αβ T cells, but not regional variation for thymus-independent αβ T cells and γδ T cells (both thymus-dependent and independent).

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## Materials and Methods

**Mice.** Specific-pathogen-free (SPF) BALB/c +/+ (+/+), BALB/c *nu*/+ (*nu*/+), and BALB/c *nu*/*nu* (*nu*/*nu*) male mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). These mice were certified by the vendor to be free of the following pathogens: Sendai virus, mouse hepatitis virus, ectromelia virus, pneumonia virus of mice, lymphocytic choriomeningitis virus, mouse adenovirus, *Pseudomonas aeruginosa*, *Salmonella* spp., *Pasteurella pneumotropica*, *Escherichia coli* O115 a, c: K (B), *Corynebacterium kutscheri*, *Mycoplasma* spp., Tyzzer's organism, *Syphacia* spp., *Giardia* spp., *Spironucleus* spp., *Trichomonas* spp., and *Entamoeba* spp. Mice were adapted for at least 2 weeks in our SPF animal facility until studied. At our facility, tests for *C. kutscheri*, *M. pulmonis*, ectromelia virus, mouse hepatitis virus, *S. typhimurium*, Sendai virus, and Tyzzer's organism are routinely performed. Evidence of infection was not found in our animals. The room temperature, relative humidity, ventilation, and lighting were 23 ± 2°C, 55 ± 5%, 15 times/h, and 14 h light (8 a.m. to 10 p.m.)/10 h dark (10 p.m. to 8 a.m.) cycle, respectively. Mice were kept in metal cages with wood chip bedding (White Flake, Charles River Japan, Inc., Yokohama, Japan) and were fed commercial pellets (MF, Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum. The Laboratory Animal Use and Care Committee of the Faculty of Agriculture, the University of Tokyo, approved the study.

**Experimental design.** Mice were studied at 11 to 12 weeks of age. Sampling of the small intestine was performed between 1 and 2 p.m. to minimize diurnal variation (11). The mice were euthanized by inhalation of an overdose of ether; then the small intestine was taken from each mouse, and the length of the intestine was measured. The intestine was then divided into three (proximal, middle and distal) parts, each of the same length. The IELs were prepared from each part separately according to the method described by us, with a minor modification (9, 11). Briefly, Peyer's patches and mesentery were carefully removed from the intestine

and each part of the small intestine was first cut longitudinally, then minced into small pieces. The pieces were placed in 25 ml of Joklik-modified minimum essential medium (JMM; GIBCO BRL, Grand Island, N.Y.) containing 1 *mM* EDTA·4Na and 2% fetal bovine serum (FBS; JRH Biosciences, Lenexa, Kans.; [JMM-EDTA]) with shaking at 37°C for 10 to 20 min in a 50-ml centrifuge tube. The supernatant was removed, 25 ml of fresh JMM-EDTA was added, and the incubation and removal of the supernatant steps were repeated. The supernatant was passed through a cotton gauze column, centrifuged, and resuspended in RPMI 1640 medium (Nissui Co., Tokyo, Japan) containing 5% FBS, DNase I (0.05 mg/ml; Boehringer Mannheim, Tokyo, Japan) and collagenase (0.5 mg/ml; Wako Pure Chemical Industries, Ltd., Tokyo, Japan). The suspension was incubated at 37°C for 5 min in a shaking water bath. After the suspension was washed with RPMI 1640 medium containing 5% FBS, the number of IELs in the resultant cell suspension was counted by use of a hemocytometer. Contaminated red blood cells and enterocytes, which were recognized on the basis of size and morphology, were not counted. The suspension was subjected to Percoll (Pharmacia, Tokyo, Japan) density-gradient centrifugation, and the sediment of cells separating at the 40% Percoll gradient were IELs. Isolation of IELs was performed, using small intestines pooled from two or five *nu*/+ and *nu*/*nu* mice, because the numbers of IELs recovered from single mice were insufficient for flow cytometric analysis.

**Flow cytometry.** The IELs were fluorescence labeled with the following antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD3ε (145-2C11), phycoerythrin (PE)-conjugated anti-mouse CD19 (1D3), PE-conjugated anti-mouse CD4 (RM4-5), Cy-Chrome-conjugated anti-mouse CD4 (RM4-5), FITC-conjugated anti-mouse CD8α (53-6.7), FITC-conjugated anti-mouse CD8β (53-5.8), Cy-Chrome-conjugated anti-mouse β T-cell receptor (TCR) (H57-597), and PE-conjugated anti-mouse γδ TCR (GL3) monoclonal antibodies (mAb) that were specific for mouse antigens and were purchased from PharMingen (San Diego, Calif.). Anti-mouse CD16/32 (FcγIII/II receptor) mAb purified from tissue culture supernatant of the hybridoma clone 2.4G2 (HB-197, American Type Culture Collection) was used to minimize nonspecific binding of the conjugated mAbs. After being fluorescence labeled, the cells were fixed with 5% formalin in phosphate buffered saline. Acquisition of at least 10,000 cells in total was performed, using a fluorescence-activated cell scanner (FACScan flow cytometer, Becton-Dickinson, Mountain View, Calif.), and the analysis was performed by use of the Cell Quest program (Becton-Dickinson).

**Data analysis.** Statistical analysis was carried out by use of an unpaired *t*-test between the percentages of each subset of the three parts of the small intestine in each type of mice.

## Results

To determine whether the number of IELs in various parts of the small intestine is influenced by presence of the thymus, we determined the number of IELs from *nu*/*nu* mice and *nu*/+ and +/+ controls. The total numbers of IELs in *nu*/+ and *nu*/*nu* mice were about a fifth that in +/+ mice (Fig. 1). In addition, there were fewer IELs in the distal part, compared with the proximal and middle parts in each type of mice ($P < 0.01$, Fig. 1). The percentages of IELs were significantly different among three parts (proximal > middle > distal) in each type of mice ($P < 0.01$, Table 1).

Because IEL numbers varied in *nu*/+ and +/+ mice, we could not use changes in cell numbers to assess the role of the thymus in repopulating various parts of the small intestine. Accordingly, we assessed the cell composition of IELs by use of flow cytometry in three parts of the small intestine in +/+, *nu*/+, and *nu*/*nu* mice. We first assessed the percentage of IELs that were T cells, B cells, and non-T non-B cells by use of flow cytometry. The presence of B cells in the IEL preparation indicated contamination of the IEL preparation by lamina propria cells (12), and few (< 1%) B cells ($CD3\varepsilon^-$, $CD19^+$) were detected in any IEL preparation (Fig. 2). In +/+ mice, the increase in the percentage of T cells ($CD3\varepsilon^+$, $CD19^-$) reached

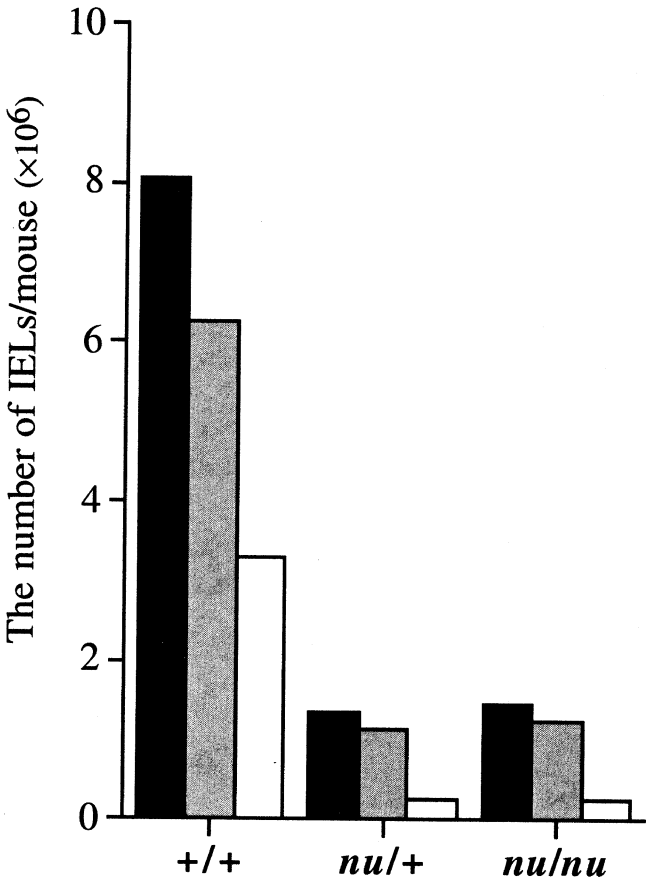


**Figure 1.** Numbers of intraepithelial lymphocytes (IELs) in each part of the small intestine.
$P < 0.01$ among all parts in +/+ mice.
$P < 0.01$ between the proximal and the distal parts and between the middle and the distal parts in *nu*/+ and *nu*/*nu* mice.

**Table 1.** Percentages of intraepithelial lymphocytes (IELs) in each part of the small intestine in +/+, *nu*/+, and *nu*/*nu* mice

| Mice | Proximal | Middle | Distal |
|---|---|---|---|
| +/+ | 45.80±1.91% | 35.48±3.59%* | 18.72±2.14%*,† |
| *nu*/+ | 49.44±2.90% | 40.95±2.17%† | 9.61±1.70%*,† |
| *nu*/*nu* | 49.38±2.24% | 42.29±1.63%† | 8.33±2.43%*,† |

*$P < 0.01$, compared with the proximal part.
†$P < 0.01$, compared with the middle part.
Data are expressed as mean ± SD.

statistical significance ($P < 0.05$), but the magnitude of the change was modest (Fig. 2; 94.1 ± 1.2%, 94.2 ± 1.9%, and 96.9 ± 0.6%, respectively). In addition, the percentage of IELs in +/+ mice that were non-T non-B cells ($CD3\varepsilon^-$, $CD19^-$) was modestly but significantly ($P < 0.05$) higher in the proximal and middle parts than in the distal part (Fig. 2; 5.6 ± 1.2%, 5.5 ± 2.0%, and 2.5 ± 0.5%, respectively). Such regional differences were not found in the three small intestinal parts of *nu*/+ and *nu*/*nu* mice. There was, however, marked and significant ($P < 0.01$) difference in the percentage of IELs that were T cells in *nu*/*nu* mice, compared with *nu*/+ and +/+ controls. To determine whether the non-T non-B cells in IELs expressed cytoplasmic CD3 and, thus, were T cell precursors, we fixed and permeabilized the IELs and assessed CD3 expression by use of flow cytometry. In +/+ and *nu*/*nu* mice, virtually all (> 98%) of the IELs were $CD3\varepsilon^+$.

To determine whether the fraction of αβ T cells and γδ T cells in T cell population in the various parts of the small intestine is influenced by presence of the thymus, we assessed by use of flow cytometry, the percentage of IEL T cells from +/+, *nu*/+, and *nu*/*nu* mice that were TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$. The percentage of T cells ($CD3\varepsilon^+CD19^-$) was approximately equal to the sum of αβ T cells and γδ T cells, indicating we were accounting for all T cells. The percentage of T cells that were TCR$\alpha\beta^+$ was significantly ($P < 0.05$) higher in the distal part, compared with the proximal and middle parts in +/+ and *nu*/+ mice (Fig. 3: 69.0 ± 1.7%, 69.1 ± 2.5%, and 86.3 ± 2.5%, respectively, in +/+ mice; 42.8 ± 3.8%, 39.2 ± 5.5%, and 55.5 ± 9.8%, respectively, in *nu*/+ mice). In contrast, the percentage of T cells that were TCR$\alpha\beta^+$ was similar in the proximal, middle, and distal parts of the small intestine of *nu*/*nu* mice. There also were marked, significant ($P < 0.01$) differences between the percentage of T cells that were TCR$\alpha\beta^+$ in each of the three groups of mice, with αβ T cells comprising the largest portion of the T-cell population in +/+ mice and the lowest portion in *nu*/*nu* mice.

To determine whether the composition of subsets in αβ T cells in various parts of the small intestine is influenced by presence of the thymus, we analyzed αβ $TCR^+$ IELs from +/+, *nu*/+, and *nu*/*nu* mice for their expression of CD4, CD8α, and CD8β by use of flow cytometry. The differences in proportions of each subset in αβ T cells were most marked in +/+ mice (Fig. 4). The percentages of $CD4^+$ and $CD8^-$ T cells and $CD4^+$, $CD8\alpha\alpha^+$ DP T cells were significantly ($P < 0.05$) higher in the distal than in the proximal part in +/+ mice (Fig. 4). In contrast, the percentage of $CD4^-$, $CD8\alpha\alpha^+$ T cells was highest in the proximal and lowest in the distal part in +/+ mice (Fig. 4, $P < 0.01$ among the three parts). In *nu*/+ mice, the trend was similar for +/+ mice, but the only significant ($P < 0.05$ between the proximal and the distal parts) difference was in the percentage of $CD4^-$, $CD8\alpha\alpha^+$ T cells, which was significantly higher in the proximal, compared with the distal part. In *nu*/*nu* mice, significant differences were not found in any subsets.

To determine the composition of subsets in γδ $TCR^+$ IELs for their expression of CD4, CD8α, and CD8β by use of flow cytometry.

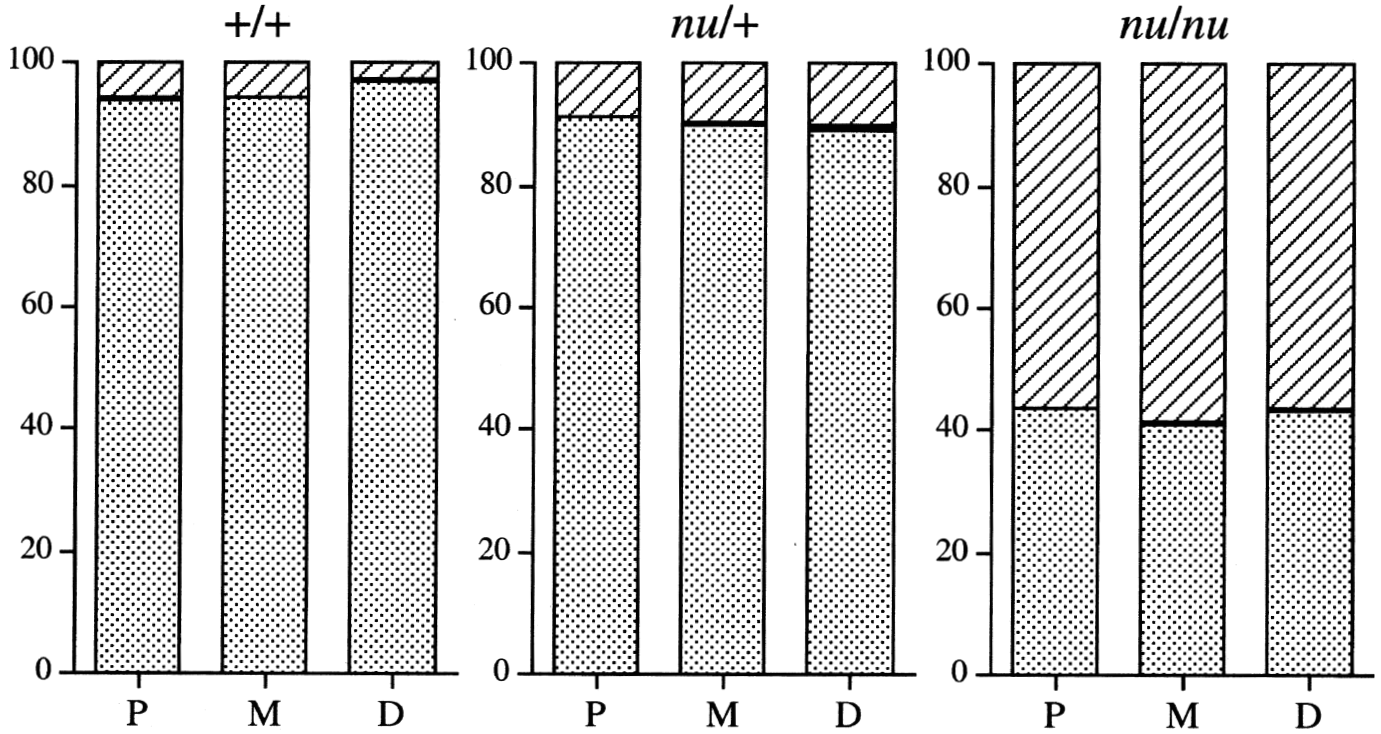


**Figure 2.** Percentages of each IEL subset, compared with total IELs in each part of the small intestine.
P = proximal, M = middle, and D = distal.

T cells: $P < 0.01$ between the proximal and the distal parts, and $P < 0.05$ between the middle and the distal parts in +/+ mice.
Non-T non-B cells: $P < 0.01$ between the proximal and the distal parts, and $P < 0.05$ between the middle and the distal parts in +/+ mice.

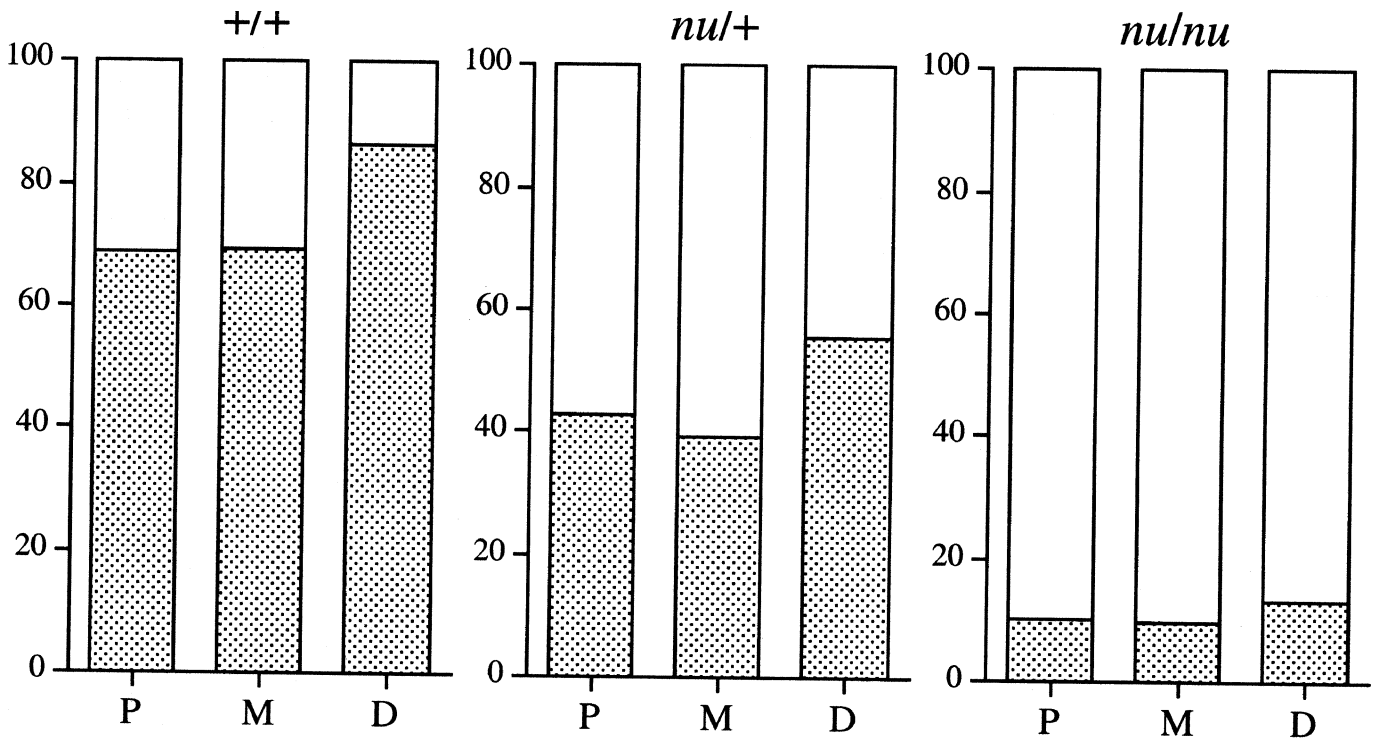


**Figure 3.** Percentages of αβ T and γδ T cells, compared with all T cells in each part of the small intestine.
P = proximal, M = middle, and D = distal.

αβ T cells and γδ T cells: $P < 0.01$ between the proximal and the distal parts and between the middle and the distal parts in +/+ mice, and $P < 0.05$ between the proximal and the distal parts and between the middle and the distal parts in *nu*/+ mice.

There were no significant differences in γδ T cell subsets among the three types of mice (Fig. 5).

## Discussion

The effects of the thymus on the regional variations of IELs were examined in the small intestine of +/+, *nu*/+, and *nu*/*nu* mice. Our observation that the total number of IELs in *nu*/*nu* mice is less than a fifth that in +/+ mice confirms an earlier report (13), but the markedly decreased number of IELs in *nu*/+ mice has, to the best of our knowledge, not been reported. It is well known that *nu*/+ mice are not equivalent immunologically to +/+ mice (14); *nu*/+ mice have decreased thymic weight (14), decreased numbers of stem cells in the bone marrow (15, 16), and lymphopenia (15), compared with +/+ mice. However, the capacity to support the local development of IELs in *nu*/*nu* mice seems to be normal because all subsets are constituted in *nu*/*nu* mice by grafting of the thymus from a +/+ mouse (17). Taken together, the significant decrease in numbers of IELs in *nu*/+ and *nu*/*nu* mice, compared with +/+ controls, is most likely due to the decrease of stem cells in the bone marrow of *nu*/+ and *nu*/*nu* mice, although bone marrow transplantation experiments are needed to confirm this point in future study. Thus, changes in cell numbers cannot be used to determine the thymus-dependent effects on IELs.

The number of IELs isolated from portions of the small intestine decreased from the proximal to the distal part in all three groups of mice (+/+, *nu*/+. and *nu*/*nu*), indicating regional variation in the number of IELs even in the absence of a thymus. We previously reported that, in euthymic mice, the number of IELs in each part of the intestine was not related to bacterial load in that portion of the intestine (9, 10). Indeed, the number of intestinal bacteria in the distal part is about 100-fold higher than that in the proximal part (6). Rather, the length of the villi may determine the number of IELs present in each part of the small intestine (9, 10). Similar to those in euthymic mice, the percentages of IELs isolated from each part of the *nu*/*nu* small intestine were highest in the proximal part of the small intestine, indicating that bacterial load did not determine thymus-independent T-cell population of regions of the small intestine.

The vast majority (> 90%) of IELs in each part of the small intestine of +/+ and *nu*/+ mice were T cells, and this percentage did not vary greatly among the three parts. In contrast, the percentage of IELs in *nu*/*nu* mice that were T cells was only about 40%, and this percentage was similar in all three parts. However, virtually all the non-T, non-B cells were T-cell precursors because they expressed intracytoplasmic CD3ε. There are several reports that presence of a thymus affects extrathymic development of T cells (18). Results of the study reported here also support this theory because of the high number of T cell-precursor cells observed only in *nu*/*nu* mice. Moreover, despite a lower thymic weight than that in +/+ mice, T-cell development seems to be normal in *nu*/+ mice because the majority of T cells are mature and the percentage of IELs that are T cells is of the same magnitude (> 90%) in +/+ and *nu*/+ mice. Again, the difference in numbers argues that lower stem cell numbers are responsible for the leukopenia in *nu*/+ mice.

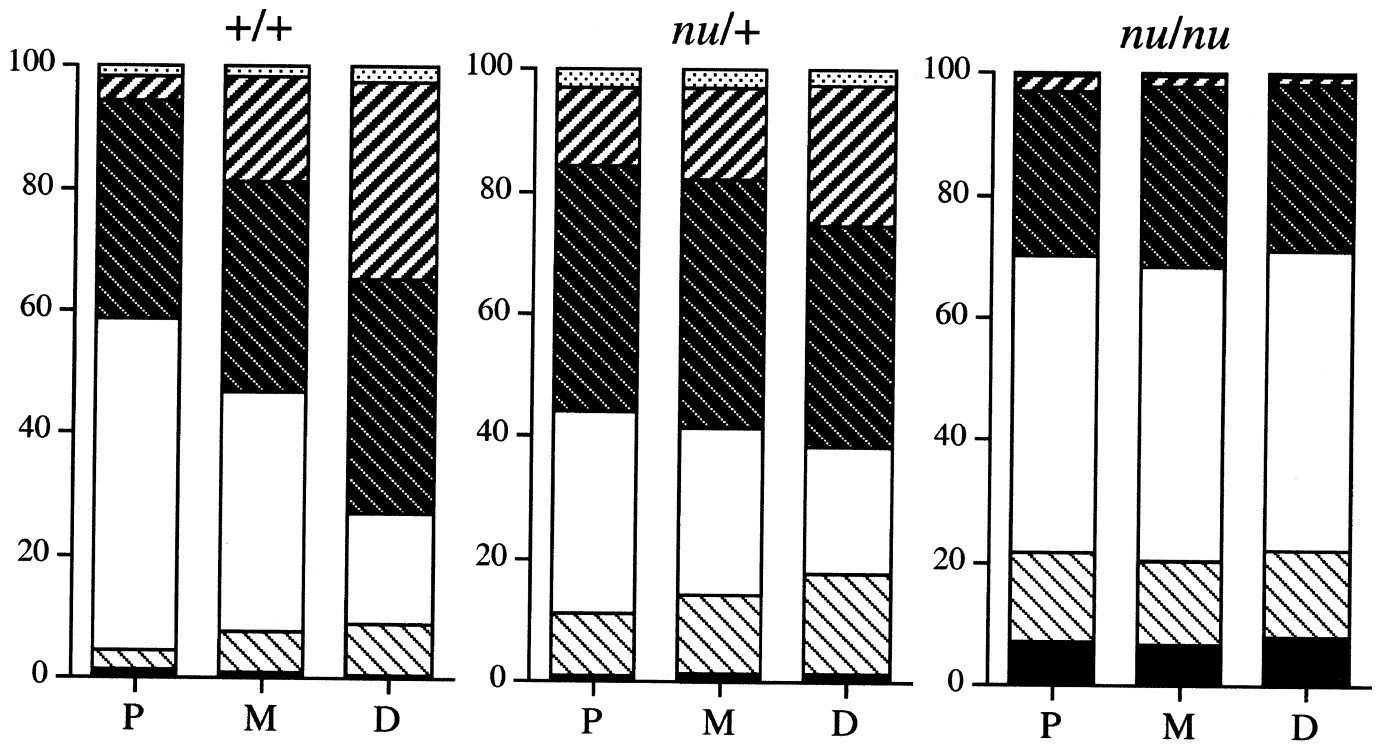


**Figure 4.** Percentages of each subset of αβ T cells, compared with all αβ T cells in each part of the small intestine.
P = proximal, M = middle, and D = distal.

double-negative T cells
CD4 T cells
CD8αα T cells
CD8αβ T cells
CD4CD8αα double-positive T cells
CD4CD8αβ double-positive T cells

DN T cells: $P < 0.01$ between the proximal and the distal parts and between the middle and the distal parts in +/+ mice.
CD4 T cells: $P < 0.01$ between the proximal and the middle parts and between the proximal and the distal parts in +/+ mice.
CD8αα T cells: $P < 0.01$ among all parts in +/+ mice, and $P < 0.05$ between the proximal and the distal parts in *nu*/+ mice.
CD4CD8αα DP T cells: $P < 0.01$ between the proximal and the middle parts and between the proximal and the distal parts, $P < 0.05$ between the middle and the distal parts in +/+ mice.

In contrast to minimal changes observed in the percentage of IELs that were T cells, the percentage of T cells that expressed TCRαβ was significantly higher in the distal part than in the proximal and middle parts in +/+ and *nu*/+ mice, whereas there was no difference in these percentages in *nu*/*nu* mice. We, therefore, conclude there is small intestinal regional variation in the αβ T cell subset only if the thymus is present.

In euthymic mice, the intestinal αβ T cell population expands locally on recognition of foreign antigens (19), including those of gastrointestinal microflora. This observation together with the 100-fold higher numbers of bacteria in the distal portion of the small intestine suggests that the αβ T cell subset expands locally and is most pronounced in the distal part of the intestine. In contrast to that in euthymic mice, there was no preferential expansion of the αβ T cell subset in the distal portion of the intestine of athymic mice. These observations suggest that αβ T cells, especially in the distal portion, are mainly derived from the thymus and that extrathymically derived αβ T cells may not recognize intestinal bacterial antigens or their proliferative activity may be low.

In the αβ T cell population, the percentage of CD8αα T cells was highest in the proximal part of the +/+ mouse small intestine, whereas the percentages of $CD4^+$, $CD8^-$, and $CD4^+CD8\alpha\alpha^+$ DP T cells were lowest. Although it was less pronounced, a similar pattern was found in *nu*/+ mice. The $CD8\alpha\alpha^+$ T cells, which largely bear self-reactive forbidden TCRs, contain FcRγ chains instead of CD3ζ chains in their TCR-CD3 complex, and mature in an IL-2Rβ-dependent manner, are proposed to develop extrathymically (20, 21). On the other hand, $CD4^+$ T cells migrate from the thymus to the intestine, and express CD8αα homodimers in response to the intestinal bacteria to become $CD4^+CD8\alpha\alpha^+$ DP T cells (22, 23). Therefore, $CD4^+$, $CD8^-$, and $CD4^+CD8\alpha\alpha^+$ DP T cells are postulated to belong to the same lineage and be thymus-derived. These findings predict that $CD4^+$, $CD8^-$, and $CD4^+CD8\alpha\alpha^+$ DP T cells should not be present in athymic mice. However, we detected each component of the αβ T cell subset, including small numbers of

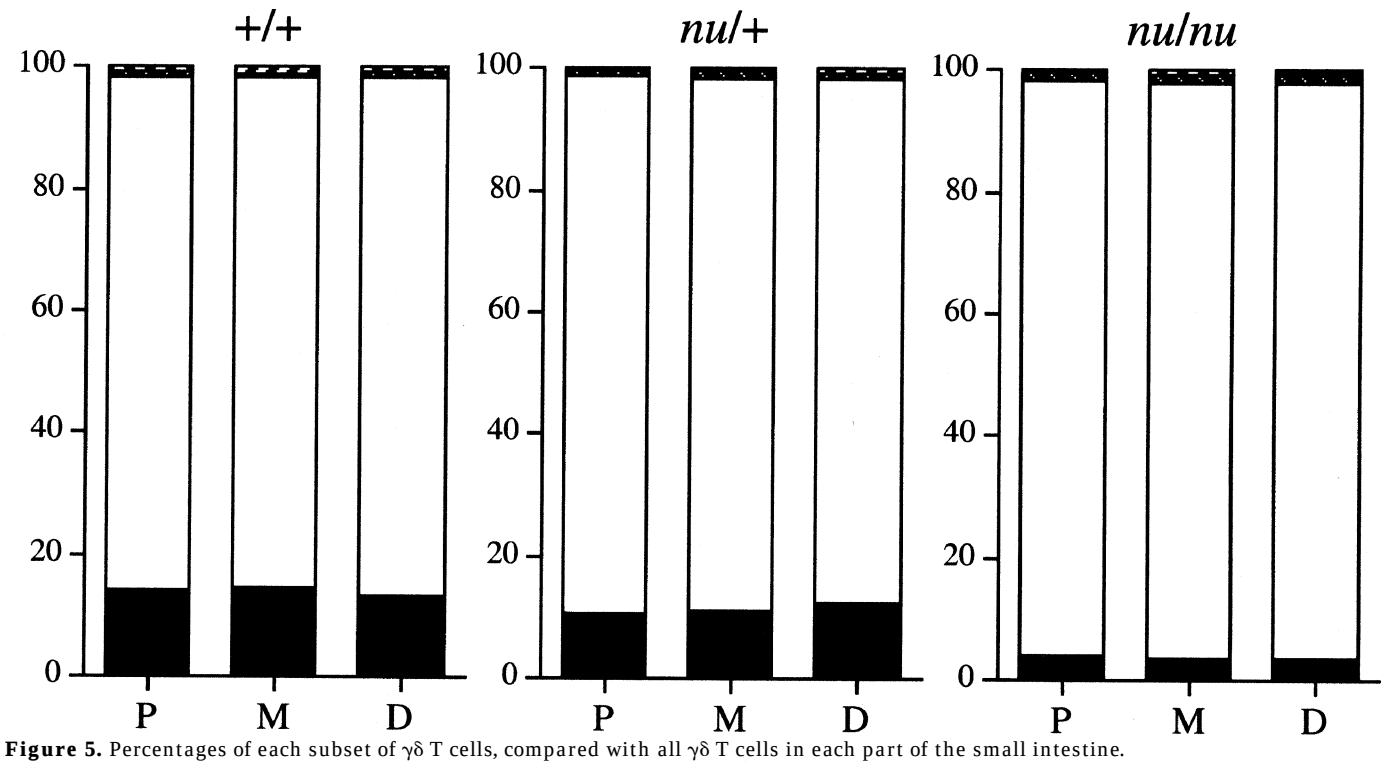


**Figure 5.** Percentages of each subset of γδ T cells, compared with all γδ T cells in each part of the small intestine. P = proximal, M = middle, and D = distal.

$CD4^+$, $CD8^-$, and $CD4^+CD8\alpha\alpha^+$ αβ T cells, in the small intestine of *nu*/*nu* mice and did not observe regional variation in the composition of the αβ T cell subset. We conclude that the vast majority of $CD4^+$, $CD8^-$, and $CD4^+CD8\alpha\alpha^+$ αβ T cells are thymus derived. The increasing percentage of $CD4^+CD8\alpha\alpha^+$ αβ T cells from the proximal to the distal portion of the small intestine mirroring the increasing numbers of intestinal bacteria also support the antigen-specific local expansion of this subpopulation of cells.

In contrast to the composition of the αβ T-cell subset, regional variations were not detected in the composition of the γδ T cell population in any of the three types of mice. This supports the previous observation that the thymic origin of γδ T cells cannot be distinguished by their phenotype (24).

In conclusion, the results of this study suggest that the decreased number of IELs, especially prominent in the distal part of the small intestine in *nu*/+ and *nu*/*nu* mice, may be caused by decreased number of bone marrow stem cells. However, regional variations in the distribution of IELs that are $TCR\alpha\beta^+$ and subsets within this population are not observed in athymic mice, whereas they are found in euthymic controls (+/+ and *nu*/+ mice). Regional variation was not detected for the composition of the γδ T-cell subset.

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## References

1. **Brown, T. A.** 1996. Immunity at mucosal surfaces. Adv. Dent. Res. **10:**62-65.
2. **Guy-Grand, D., M. Malassis-Seris, C. Briottet, and P. Vassalli.** 1991. Cytotoxic differentiation of mouse gut thymodependent and independent intraepithelial T lymphocytes is induced locally. Correlation between functional assays, presence of perforin and granzyme transcripts, and cytoplasmic granules. J. Exp. Med. **173:**1549-1552.
3. **Fan, J. Y., C. S. Boyce, and C. F. Cuff.** 1998. T-helper 1 and T-helper 2 cytokine responses in gut-associated lymphoid tissue following enteric reovirus infection. Cell. Immunol. **188:**55-63.
4. **Hummel, K. P., F. L. Richardson, and E. Fekete.** 1975. Anatomy, p. 247-307. *In* E. L. Green (ed.), Biology of the laboratory mouse (2nd ed.). Dover Publications Inc., New York
5. **Ware, J. A., and C. K. Svensson.** 1996. Longitudinal distribution of arylamine N-acetyltransferases in the intestine of the hamster, mouse, and rat. Evidence for multiplicity of N-acetyltransferases in the intestine. Biochem. Pharmacol. **52:**1613-1620.
6. **Smith, H. W.** 1965. Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Pathol. Bacteriol. **89:**95-122.

7. **Sidhu, N. K., G. M. Wright, R. J. Markham, W. P. Ireland, and A. Singh.** 1992. Quantitative regional variation in the expression of major histocompatibility class II antigens in enterocytes of the mouse small intestine. Tissue Cell **24:**221-228.
8. **Amano, M., J. R. McGhee, M. J. McCutcheon, K. Fujii, and K. H. Kiyono.** 1993. Application of the ILISPOT-IDIP system for the enumeration of different sizes of IgA spot forming cells in the murine small and large intestine. J. Immunol. Methods **164:**79-90.
9. **Suzuki, H., K. I. Jeong, T. Okutani, and K. Doi.** 2000. Regional variations in the number and subsets of intraepithelial lymphocytes in the mouse small intestine. Comp. Med. **50:** 39-42.
10. **Suzuki, H., K. I. Jeong, T. Okutani, and K. Doi.** 2000. Regional variations in the distributions of small intestinal intraepithelial lymphocytes (IELs) in three inbred strains of mice. J. Vet. Med. Sci., in press.
11. **Suzuki, H., S. Shibata, T. Okutani, M. Suzuki, M. Nakayama, T. Nishimura, and K. Doi.** 1999. Diurnal changes in intraepithelial lymphocytes (IELs) in the small intestine of mice. Exp. Anim. **48:** 115-118.
12. **Mosley, R. L., D. Styre, and J. R. Klein.** 1990. CD4+CD8+ murine intestinal intraepithelial lymphocytes. Int. Immunol. **2:**361-365.
13. **Bandeira, A., S. Itohara, M. Bonneville, O. Burlen-Defranoux, T. Mota-Santos, A. Coutinho, and S. Tonegawa.** 1991. Extrathymic origin of intestinal intraepithelial lymphocytes bearing T-cell antigen receptor γδ. Proc. Natl. Acad. Sci. USA. **88:**43-47.
14. **Bartak, A., M. Bokorova, Z. Rychter, and M. Holub.** 1978. The thymus of the *nu*/+ hybrid. Folia Biol. **24:**419-20.
15. **Dolenska, S., M. Holub, and B. Mandi.** 1978. Bone marrow stem cell potential in mice bearing the nu gene. Folia Biol. **24:**421-423.
16. **Holub, M., E. Necas, and Z. Jiraskova.** 1998. Essential defects of athymic nude mice affects also the *nu*/+ heterozygotes. Folia Microbiol. **43:**491-492.
17. **Lin, T., G. Matsuzaki, H. Yoshida, H. Kenai, K. Omoto, M. Umesue, C. Singaram, and K. Nomoto.** 1996. Thymus ontogeny and the development of TCR αβ intestinal intraepithelial lymphocytes. Cell. Immunol. **171:**132-139.
18. **Lin, T., G. Matsuzaki, H. Kenai, and K. Nomoto.** 1995. Extrathymic and thymic origin of murine IEL: are most IEL in euthymic mice derived from the thymus? Immunol. Cell Biol. **73:**469-473.
19. **Umesaki, Y., H. Setoyama, S. Matsumoto, and Y. Okada.** 1993. Expansion of αβ T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. Immunology **79:**32-37.
20. **Rocha, B., P. Vassalli, and D. Guy-Grand.** 1991. The Vβ repertoire of mouse gut homodimeric α CD8+ intraepithelial T cell receptor α/β+ lymphocytes reveals a major extrathymic pathway of T cell differentiation. J. Exp. Med. **173:**483-486.
21. **Page, S. T., L. Y. Bogatzki, J. A. Hamerman, C. H. Sweenie, P. J. Hogarth, M. Malissen, R. M. Perlmutter, and A. M. Pullen.** 1998. Intestinal intraepithelial lymphocytes include precursors committed to the T cell receptor αβ lineage. Proc. Natl. Acad. Sci. USA. **95:**9459-9464.
22. **Takeuchi, M., H. Miyazaki, K. Mirokawa, T. Yokokura, and Y Yoshikai.** 1993. Age-related changes of T cell subsets in intestinal intraepithelial lymphocytes of mice. Eur. J. Immunol. **23:**409-1411.
23. **Lin, T., G. Matsuzaki, H. Kenai, and K. Nomoto.** 1995. Extrathymic and thymic origin of murine IEL: are most IEL in euthymic mice derived from the thymus? Immunol. Cell Biol. **73:**469-473.
24. **Lin, T., G. Matsuzaki, H. Kenai, K. Kishihara, S. Nabeshima, W. P. Fung-Leung, T. W. Mak, and K. Nomoto.** 1994. Characteristics of fetal thymus-derived T cell receptor γδ intestinal intraepithelial lymphocytes. Eur. J. Immunol. **24:**1792-1798.