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Age-dependent changes in intraepithelial lymphocytes (IELs) of the small intestine, cecum, and colon from young adult to aged mice

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ABSTRACT

We previously reported the regional differences in the IELs present in the proximal (P), middle (M), and distal (D) parts of the small intestine, cecum (Ce), and colon (Co) of mice. In this study, we investigated the age-dependent changes in the regional differences of IELs from young adult to aged mice. In this experiment, 3-, 6-, 12-, 18-, and 24-month-old mice were examined. IELs were separately isolated from 5 parts of the intestines and analyzed by flow cytometry. Regional differences in the number and phenotype of IELs showed the same trends in all age groups. The number of IELs was highest in 6-month-old mice and then gradually decreased with age. As to IEL subsets, age-related changes were not seen except for a few subsets among the age groups. We conclude that age-related decreases in IELs in mouse small intestine may be one of the aging phenomena of the intestinal immune system. Such age-related decreases in IELs may be concerned with the increased liability to intestinal infections in the elderly.

1. Introduction

IELs, which reside in the epithelial layer of the intestine, are one of the components of the intestinal immune system and may act as a first line of defense against intestinal pathogens. We have studied the regional differences in IELs of mouse small intestine for many years (Suzuki et al., 2000a,b, 2001a,b, 2002a,b; Suzuki and Yamamoto, 2006). In our recent paper, we expanded our previous findings of IELs in the small intestine to those in the entire intestine and reported that significant differences were found mainly between the small and large intestines, especially in the composition of the subset comprising αβ T cells and γδ T cells, although some differences were also found among the P, M, and D parts of the small intestine and between the Ce and Co (Suzuki, 2009). We concluded that the differences between IELs in the small and large intestines are discontinuous and that IELs distribute to the small and large intestines in different ways.

The function of the immune system is known to decline in the aged humans and animals. This is called "aging of the immune system", also referred as "immuno-senescence" (Aw et al., 2007). In the mucosal immune system, aging was also reported in B-cell responses and T-cell regulatory mechanisms (Cripps and Gleeson, 2005). As to IELs, age-related changes in T cell subsets were reported in aged mice (Takeuchi et al., 1993).

In this study, we examined the number and phenotypic composition of IELs in the P, M and D parts of the small intestine; Ce and Co from young adult to aged mice to elucidate the age-dependent changes in IELs of the small and large intestines.

2. Materials and methods

2.1. Animals

C57BL/6 mice, purchased from Japan SLC Inc. (Shizuoka, Japan), were bred and maintained in our specific pathogen-free animal facility. The mice were kept at room temperature of 20–27 °C and relative humidity of 30–70%, with 12-h light (08:00–20:00)–12-h dark (20:00–08:00) cycle. The mice were housed in plastic cages with wood chip bedding and fed commercial pellets (CRF-1; Charles River Japan Inc., Kanagawa, Japan) and tap water ad libitum. All animal experiments were conducted with the approval of the Animal Care and Use Committee of the National Institute of Health Sciences, Japan.

2.2. Isolation of IELs

In this experiment, female mice of ages 3 months (13 ± 1 weeks), 6 months (26 ± 1 weeks), 12 months (52 ± 1 weeks), 18 months (78 ± 1 weeks), and 24 months (104 ± 1 weeks) were used. The mice showing obvious macroscopic changes, such as tumors or extensive skin lesions, were eliminated from the study. Especially in 24-month-old mice, more than 60% of the animals possessed lymphomas or other neoplastic changes in the abdominal cavity

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and were eliminated from the study. Data of 4 mice were collected in each age group. Sampling of the intestines was performed between 13:00 and 14:00 to minimize diurnal variation (Suzuki et al., 1999). The small intestine, cecum, and colon were collected from each mouse. The length of the small intestine was measured, and the small intestine was then divided into 3 parts (P, M and D) of the same length. IELs were separately isolated from each portion according to the method previously described by us, with a minor modification (Suzuki, 2009). Briefly, the mesentery and/or Peyer's patches were carefully removed from the intestines. Each portion of the intestines was cut longitudinally, washed, and then minced. The pieces were placed in 15 ml of Hanks' balanced salt solution (Sigma Chemical Co., St. Louis, MO, USA) containing 1 mM EDTA·4Na and 2% FBS (JR Scientific Inc., Woodland, CA, USA) (HBSS-EDTA) and incubated with shaking at 37 °C for 10–20 min in 50 ml centrifuge tubes. The supernatant was removed, and 15 ml of fresh HBSS-EDTA was added to each tube; the incubation and supernatant removal steps were repeated. The supernatant thus obtained was passed through a cotton gauze column, centrifuged, and resuspended in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO, USA) containing 5% FBS, 0.05 mg/ml DNase I (Roche Diagnostics GmbH, Mannheim, Germany), and 0.5 mg/ml collagenase (Wako Pure Chemical Industries Ltd., Osaka, Japan). The suspension was then incubated at 37 °C for 5 min in a water bath shaker. After washing with RPMI 1640 medium containing 5% FBS, the number of viable IELs in the resultant cell suspension was counted with a hemocytometer by the trypan blue dye-exclusion method. Contaminated RBCs and enterocytes, which were recognized on the basis of their size and morphology, were not counted. The suspension was subjected to Percoll™ (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) density-gradient centrifugation in order to separate the IELs from the epithelial cells; the sediment of cells separating at the 40% Percoll™ gradient was IELs.

2.3. Analysis of IEL subsets

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), PerCP-Cy5.5-conjugated anti-mouse NK-1.1 (PK136), 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), PE-Cy5-conjugated anti-mouse β TCR (H57-597), and PE-conjugated anti-mouse γδ TCR (GL3) monoclonal antibodies (mAbs), all purchased from BD Biosciences (Franklin Lakes, NJ, USA). Anti-mouse CD16/32 (FcγIII/II receptor) mAb purified from the tissue culture supernatant of the hybridoma clone 2.4G2 (American Type Culture Collection, HB-197) was added before the addition of the fluorescence-labeled mAbs to minimize nonspecific binding of the conjugated mAbs. After the cells were fluorescence-labeled, they were fixed with 5% formalin in phosphate-buffered saline at 4 °C for 10 min. Data acquisition and analysis were performed with a FACSCalibur flow cytometer and the Cell Quest program (BD Biosciences, Franklin Lakes, NJ, USA).

2.4. Statistics

Statistical analysis of the data was performed by using one-way analysis of variance (ANOVA), which was followed by an appropriate post hoc test (Tukey's method), for the number of IELs recovered and the percentage of each IEL subset. Statistical significance was not calculated for the subsets that comprised <1% of the gated cells in all the portions compared.

3. Results

The IELs were separately isolated from the P, M and D parts of the small intestine, the Ce and Co from each age group and phenotypically analyzed and statistically compared.

3.1. Number of IELs

3.1.1. Comparison among the portions within each age group of mice

In the comparison among the portions within each age group of mice, the highest number of viable IELs was recovered from the proximal part of the small intestine, followed by the middle part, distal part, and cecum, with the lowest number of IELs recovered from the colon (P > M > D > Ce > Co), in all age groups (Table 1).

3.1.2. Comparison among the age groups

In the comparison among the age groups, the number of IELs was highest in 6-month-old mice, and then the number of IELs gradually decreased with age. Statistically significant differences in the number of IELs were found for 3- and 6-month-old mice versus 12-, 18-, and 24-month-old mice in the proximal and middle parts of the small intestine. In the distal part, significant differences were found for 3- and 6-month-old mice versus 18- and 24-month-old mice (Table 1). No significant differences were found in the large intestine.

Table 1
Number of IELs in each portion of the intestine and statistical significance among the age groups of mice.

Portions	Age-groups				
	3M	6M	12M	18M	24M
P	$(5.02 \pm 0.45) \times 10^6$	$(5.21 \pm 0.26) \times 10^6$	$(4.17 \pm 0.18) \times 10^6$	$(3.70 \pm 0.27) \times 10^6$	$(3.56 \pm 0.34) \times 10^{6***}$
M	$(4.25 \pm 0.22) \times 10^6$	$(4.41 \pm 0.31) \times 10^6$	$(3.38 \pm 0.34) \times 10^6$	$(3.51 \pm 0.34) \times 10^6$	$(3.39 \pm 0.36) \times 10^{6***}$
D	$(2.08 \pm 0.25) \times 10^6$	$(2.19 \pm 0.34) \times 10^6$	$(1.72 \pm 0.28) \times 10^6$	$(1.33 \pm 0.16) \times 10^6$	$(1.35 \pm 0.16) \times 10^{6***}$
Ce	$(5.00 \pm 0.82) \times 10^5$	$(5.58 \pm 1.03) \times 10^5$	$(4.08 \pm 0.83) \times 10^5$	$(3.92 \pm 0.57) \times 10^5$	$(4.42 \pm 0.42) \times 10^5$ NS
Co	$(3.08 \pm 0.32) \times 10^5$	$(3.50 \pm 0.58) \times 10^5$	$(2.67 \pm 0.54) \times 10^5$	$(2.92 \pm 0.17) \times 10^5$	$(3.08 \pm 0.17) \times 10^5$ NS

Significances by ANOVA: $^{***}p < 0.001$

Portions	Age-groups					
	3–12M	3–18M	3–24M	6–12M	6–18M	6–24M
Inter age-group significances by Tukey's method						
P	*	***	***	**	***	***
M	*	*	*	**	**	**
D		**	**		**	**

Note: The non-indicated pairs of age-groups do not differ significantly.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.