NOTE *Anatomy*

Secondary Lymphoid Areas in Calf Ileal Peyer's Patch

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ABSTRACT. The secondary lymphoid tissues appear in sheep ileum after involution of ileal Peyer's patch (PP). However, the existence of the secondary lymphoid tissues before involution of ileal PP has not yet been studied. We examined morphological characteristics of the full length of calf ileal PP using gross and microscopic anatomical techniques. Most areas of ileal PP consisted of densely packed lymphatic follicles contained very few follicular T-cell and associated with only scant interfollicular areas. However, the proximal end of ileal PP consisted of widely dispersed lymphatic follicles contained many follicular T-cell and associated with large interfollicular areas. The histological architectures of the proximal end of ileal PP strongly resembled those of the secondary lymphoid tissue in calf.
KEY WORDS: calf, ileal Peyer's patch, secondary lymphoid tissue.

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Ruminant's gut lymphoid tissue can be broadly classified into primary and secondary lymphoid tissues according to their architecture and life history [3, 8, 10]. Ileal Peyer's patch (PP) is thought to be the primary lymphoid organ of B-cell, whose repertoire is diversified by gene conversion and/or somatic hypermutation [7, 13]. Ileal PP extends one to two meters along the terminal small intestine and contains about 100,000 lymhatic follicles [3]. Histologically, densely packed lymphatic follicles are associated with small interfollicular T-cell areas [9]. Jejunal PP is thought to be the secondary lymphoid tissue for local mucosal immunity, and the functions of this lymphoid tissue is kept throughout animal's life [3, 10, 11]. Moreover, jejunal PP lymphatic follicles are widely dispersed and separated by large interfollicular T-cell areas [3, 9–11]. At about 12 weeks old, sheep ileal PP begins to involute, and only a few lymphatic follicles remain in this area at 18 months old [8]. However, recently it has been reported that jejunal PP type tissue is found in the ileal region during involution of ileal PP, and even four years old sheep still have some jejunal PP type tissue [5]. The existence of secondary lymphoid tissue in calf ileum has not yet been studied. Therefore, we attempted to study the morphological characteristics of the full length of ileal PP using gross and microscopic methods at the most growth stage of this organ. This paper first described the existence of secondary lymphoid tissue areas in calf ileal PP before involution.

One ~ 3 months old Japanese black calves (n=6) were used, because differences between ileal and jejunal PP appeared clearly more than 1-month old, and the most growth stage of ileal PP is 1 to 3 months old [9]. To verify the full length of ileal PP, the mesenterium side of intestine was cut and opened as shown in Fig.1. The gross anatomical aspects of the full length of ileal PP and jejunal PP were shown in Fig.1A. Upper side of the intestine connected to duodenum and lower side of it connected to caecum. Although the distal portion of ileal PP occupied almost all intestinal-wall, the proximal portion of ileal PP was narrow and found in the part of intestinal-wall like jejunal PP. Therefore, jejunal PP (e), the distal end (a) and the proximal end (d) of ileal PP, and also every 10 cm interval between a to c were collected for histological analysis as shown in Fig.1B. These specimens were mounted in OCT embedding compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) on Cryomold (Sakura Finetek Japan Co., Ltd) and were frozen on dry ice, and then stored at –80°C. Cryostat sections were stained with monoclonal antibodies (Mab) specific for CD3 (MM1A, 1,000 times dilution) and Ig μ chain (Big 73A, 1,000 times dilution), both of them were purchased from VMRD inc. (Pullman, WA, U.S.A.), using the indirect immunoperoxidase technique described by Yasuda *et al.* [11]. In brief, cryostat sections (7–9 μm thick) were air-dried on slides and fixed with ice-cold acetone for 10 min. The sections were re-hydrated in 0.1 M phosphate-buffered saline (PBS, pH7.2) and incubated with 10% normal horse serum in PBS for 30 min to block any nonspecific binding. The sections were stained with Mab for 60 min and washed three times with PBS. They were then incubated with a secondary antibody (Vector Lab., Burlingame, CA, U.S.A., 100 times dilution) absorbed with acetone powder of calf ileal PP. After incubation with the secondary antibody, endogenous peroxidase was quenched with 0.3% H_2O_2 in methanol for 30 min, followed by incubation with ABC complex (Vector Lab.) for 15 min. After the sections were rinsed three times in PBS, the reactions were made visible with metal-enhanced diaminobenzidine (Pierce, Rockford, IL, U.S.A.). Immunohistochemical staining was performed at room temperature in a moist chamber. Control staining was performed simultaneously, in which the first antibody was replaced with normal mouse IgG. No positive staining was found in the control slides (data not shown).

The distributions of $CD3^+$ cells in PP were shown in Fig. 2, and those of IgM^+ cells were shown in Fig. 3. Typical ileal PP consists of densely packed lymphatic follicles (f)

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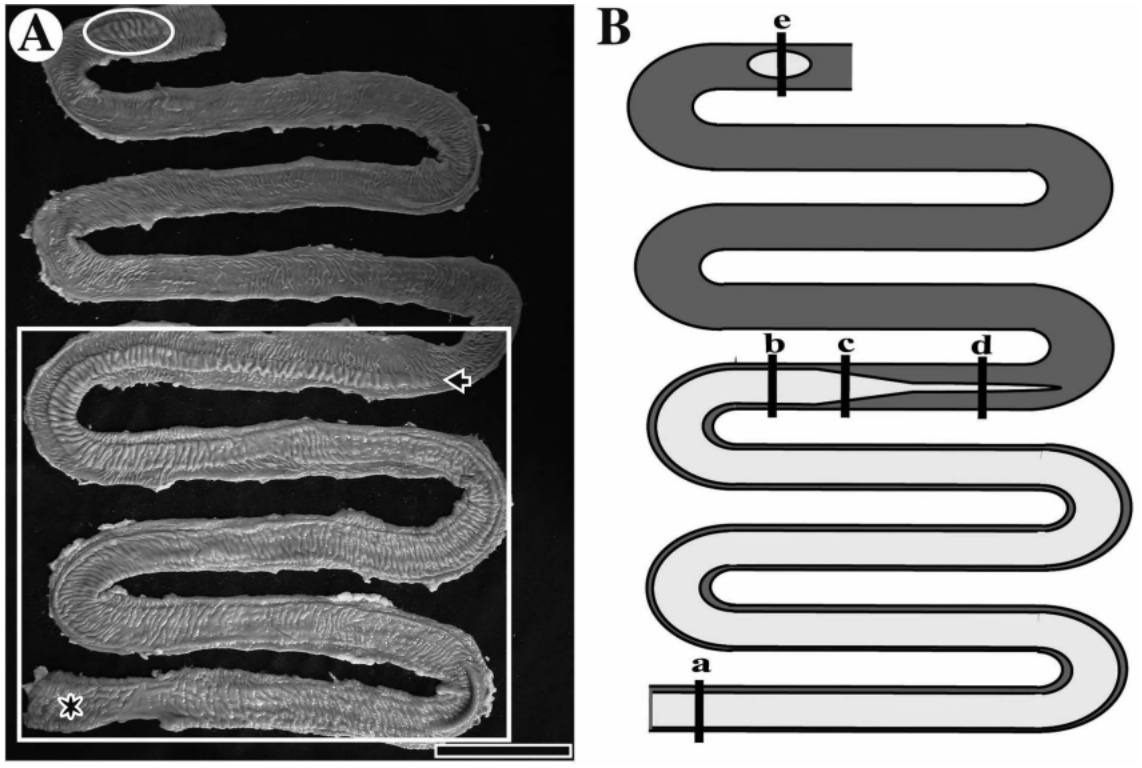

Fig. 1. Morphological aspect of the full length of ileal PP at 3 months old calf. A; The photo shows mucosal site of small intestine from the ileal opening to about four meters proximal site. The full length of ileal PP and one of jejunal PP are surrounded by rectangle and circle, respectively. Although the distal portion of ileal PP (asterisk) is wide and finds in almost all intestinal-wall, the proximal end of ileal PP (arrow) is narrow and finds in the part of intestinal-wall just like jejunal PP. Scale bar= 10 cm. B; Scheme of calf ileal and jejunal PP. The small letters (a~e) are commensurable to the capital letters (A~E) of tissue photos in Fig. 2 and Fig. 3.

and small T-cell areas as shown in Fig. 2A and Fig. 3A. Ileal PP follicles contained most IgM^+ cells as shown in Fig. 3A and very few $CD3^+$ cells as shown in Fig. 2A. $CD3^+$ cells were found in the dome region and small T-cell areas of ileal PP as shown in Fig. 2A. The tissue sections of Fig. 2A~E and Fig. 3A~E are commensurable to the location of small letter (a~e) in Fig. 1B. Between a and c portion of ileal PP as shown in Fig. 1B, we examined histological characteristics of ileal PP every 10 cm interval. Densely packed lymphatic follicles contained IgM^+ cells and very few $CD3^+$ cells, and only scant T-cell areas were found in these areas (a~c) as shown in Figs. 2B, 2C, 3B and 3C. $CD3^+$ cells were found in the dome region and small T-cell areas. Therefore, histological features of these areas were the same with those of distal end of ileal PP. However, in the proximal end of ileal PP (Figs. 2D and 3D), it consisted of wide interfollicular T-cell areas contained many $CD3^+$ cells and dispersed lymphatic follicles (f) as shown in Fig. 2D. $CD3^+$ cells were found in the follicles of this area. As shown in Figs. 2E and 3E, jejunal PP also consisted of wide interfollicular T-cell areas and dispersed lymphatic follicles (f). And many $CD3^+$ cells and IgM^+ cells were distributed in the follicles. Therefore, the histological features of the proximal end of ileal PP were remarkably similar to those of jejunal PP. In addition, all specimens of the proximal end of ileal PP using this experiment had the same histological characteristics (data not shown).

Calf ileal PP and chicken bursa of Fabricius are thought to be primary lymphoid organ for B-cell. In these organs, B-cell repertoire is generated by gene conversion and/or point mutation during ontogeny. Histologically, densely packed lymphatic follicle and the very few number of T-cell are distributed in the follicle of both primary lymphoid organ [3, 9, 12]. It has been also reported that secondary lymphoid organ such as calf jejunal PP and chicken caecal tonsil have the functions for local mucosal immunity [1, 4, 11, 12]. These organs consist of large interfollicular T-cell areas and dispersed follicular B-cell areas. In addition, many helper T-cell, IgG^+ cells and IgA^+ cells were found in these secondary lymphoid organs [9, 12]. In the chicken bursal dorsal wall connected bursal duct, the existence of diffusely infiltrated lymphoid tissue called T-dependent area is previously described [2, 6]. The bursal T-dependent area contains germinal center and mature B-cell such as IgG^+ cells and IgA^+ cells after hatching [2, 6]. Therefore, it suggested that this T-dependent area represents mucosal associated lymphoid tissue such as secondary lymphoid organ involved in the induction of immune response to antigens via chicken clo-

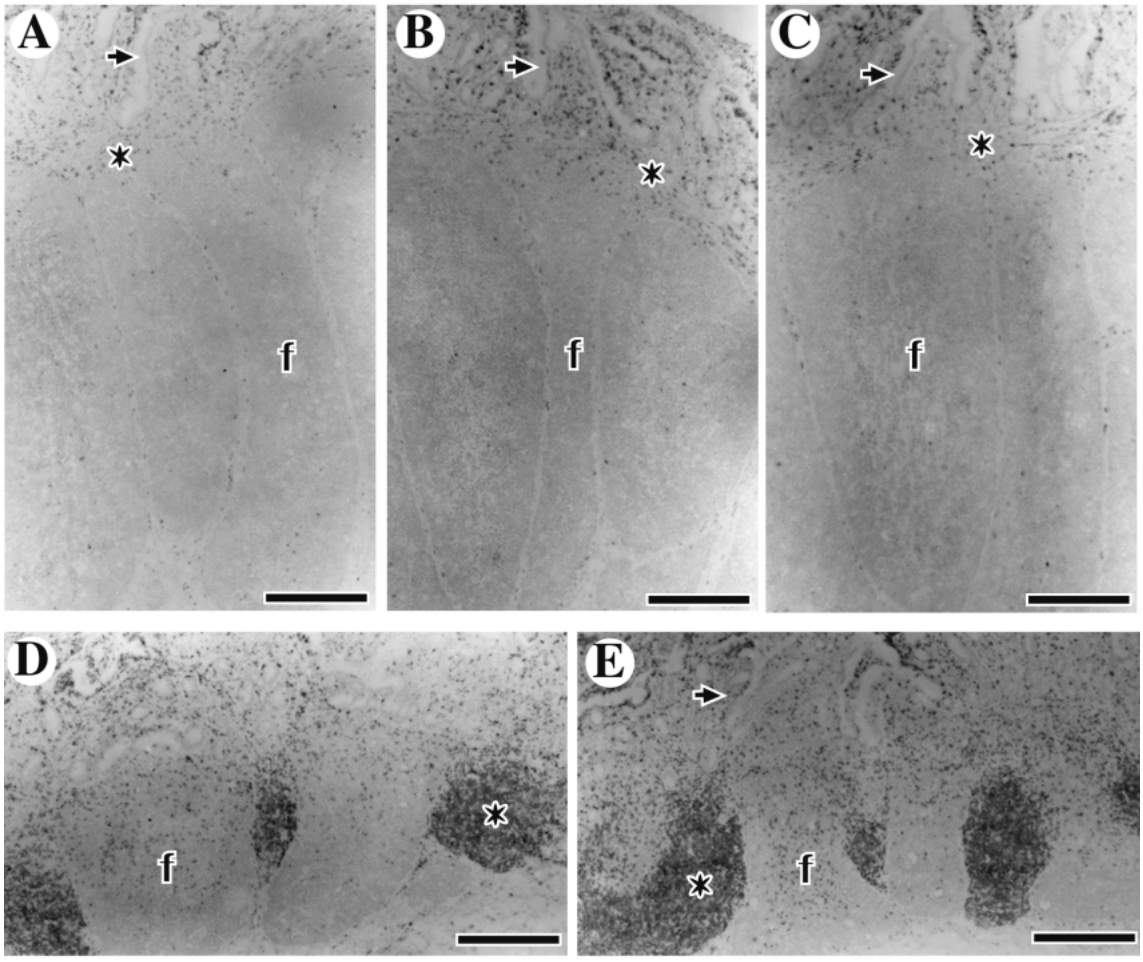

Fig. 2. The distribution of $CD3^+$ cells in ileal and jejunal PP at 1.5 months old calf. The distal end of ileal PP contains densely packed lymphatic follicles and only scant $CD3^+$ T-cell area (A). Very few $CD3^+$ cells are found in ileal PP follicles. Similar histological characteristics are observed in B and C. In the proximal end of ileal PP (D) and jejunal PP (E), these tissues consist of wide interfollicular T-cell areas and dispersed follicles. Many $CD3^+$ cells are found in these follicles. The distributions of $CD3^+$ cells in the proximal end of ileal PP (D) are strongly similar to those of the jejunal PP (E). Small letter f shows lymphatic follicle. Asterisk shows interfollicular area. Arrow shows the dome region. Scale bar= 100 μm.

aca. Consequently, the proximal end of calf ileal PP and T-dependent area of chicken bursa might be similar histological characteristics as secondary lymphoid tissues located in primary lymphoid organ. At around 1-month old calf, the migration of many helper T-cell and Ig class switching appear in jejunal PP follicles [9, 12]. Therefore, the secondary lymphatic follicles might change their characteristics under influence of environmental antigens. However, it needs further analysis for determining functions of secondary lymphoid areas in primary lymphoid organ during ontogeny.

In conclusions, the results of this study first described that the characteristics of the proximal end of ileal PP strongly resembled those of the jejunal PP.

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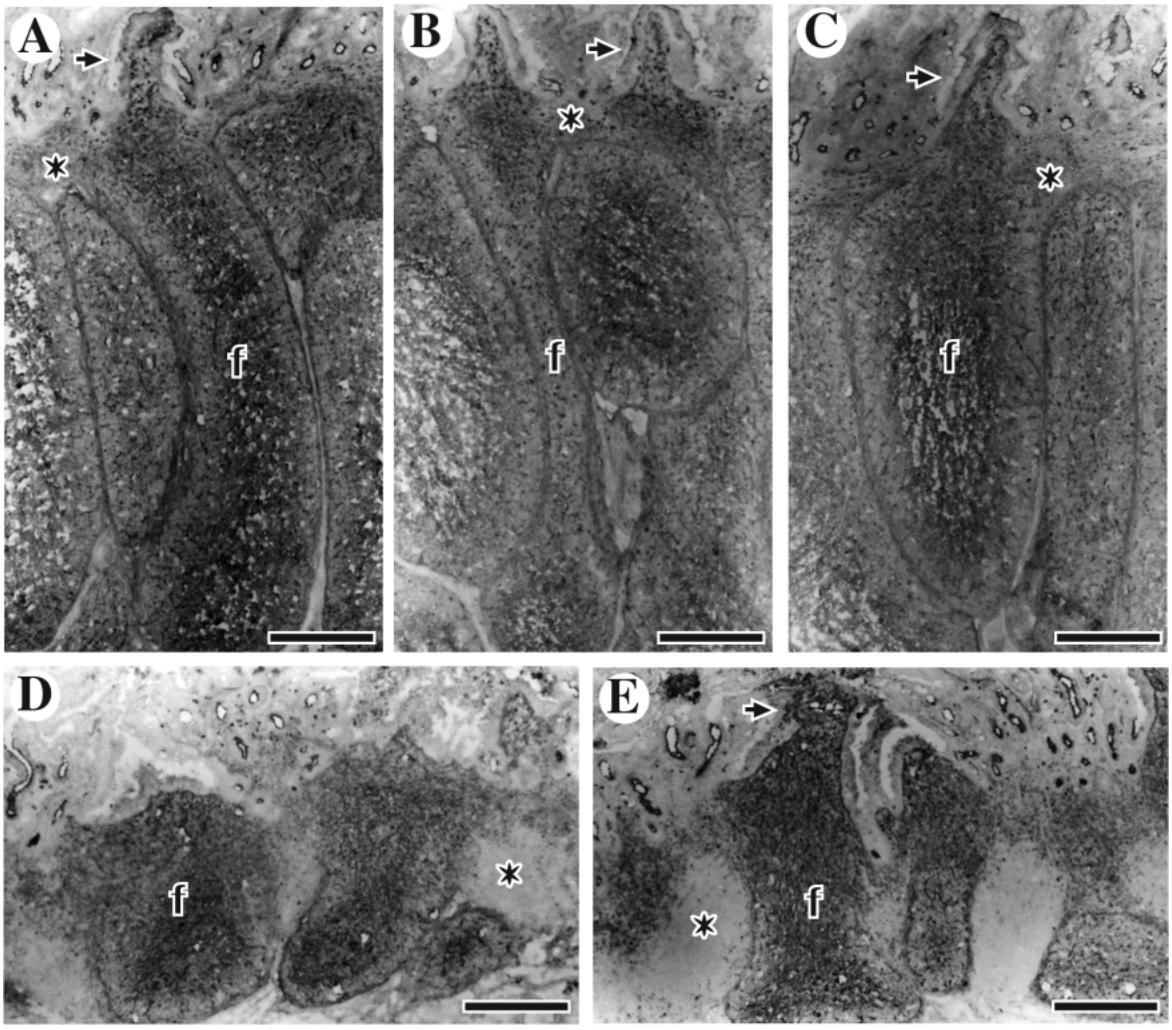

Fig. 3. The distribution of IgM^+ cells in ileal and jejunal PP at 1.5 months old calf. All sections are serial sections shown in Fig. 2. Most IgM^+ cells are distributed in the ileal and jejunal PP follicles (A~E). Ileal PP follicles are densely packed (A~C), but jejunal PP follicles were widely dispersed (E). The proximal end of ileal PP (D) consists of wide interfollicular area and dispersed follicles. The histological characteristics (D) are strongly similar to those of the jejunal PP (E). Small letter f shows lymphatic follicle. Asterisk shows interfollicular area. Arrow shows the dome region. Scale bar=100 μm.

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