Original Article

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Factors Involved in the Degeneration of Lymphoid Tissue in the Appendix

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Summary: ***Introduction*:** Studies evaluating the age-related alteration of human appendix have been reported. Although the appendix shows a degeneration of lymphoid tissues with aging, the mechanism of action remains unclear.

***Material and Methods*:** Surgically resected appendix tissues from patients with colon cancer, intestinal malrotation and ulcerative colitis (UC) were utilized for histological and flow cytometric analysis.

***Results*:** Histological analysis showed that aging may induce steatotic changes in the appendix. However, there was no clear association between appendiceal fibrosis and aging. Lymphoid follicles in the appendix may start to develop before 5 days of age, gradually mature, and eventually disappear with aging.

Flow cytometric analysis clearly identified a lymphocyte population in the appendix at 5 days, 45 and 75 years of age, and lymphoid follicles were also confirmed histologically. In contrast, lymphoid population was rarely detectable in the appendix at 79 and 80 years of age, and no lymphoid follicles were present histologically. Interestingly, cytograms from a case at 5 days of age suggested the existence of immature immune cells, as forward scatter showed an increase in cell size of the lymphocyte population.

Histological analysis in UC patients found submucosal fat in the appendix of a case 66 years of age. Lymphoid follicular formation and mucosal structure were disrupted in cases of 70 and 72 years of age. UC patients may be more susceptible to steatotic change. Cytograms from appendices of UC patients also supported these histological findings.

Our study confirms previous results that lymphoid tissues in the appendix degenerate over time, and proposes that inflammatory insult may facilitate the degenerative process in patients with UC.

Key words appendix, degeneration, steatosis, fibrosis, lymphoid follicle, flow cytometry

INTRODUCTION

Although the human appendix represents a well-organized lymphoid tissue composed of lymphoid follicles [1,2], it has long been considered a vestigial organ. However, the immune role of the appendix is becoming increasingly apparent [3]. The appendix is where a part of gut associated lymphoid tissue (GALT) develops from birth [4] through interaction with intestinal flora [5]. Interestingly, appendectomy performed in T cell receptor (TCR) α deficient mice, a model of UC, before, but not after, 3 weeks of age, was proposed to suppress the development of colitis [6]. This experimental finding has since been supported in humans showing a negative association between development of UC and prior appendectomy before the age of 20 [7]. These results raised the possibility that the appendix may provide a priming site for potentially pathogenic immune cells. Alternatively, the appendix has recently been shown to represent the main site of production of IgA [8], thus suggesting a potential protective role against infection.

Human appendiceal lymphoid follicles start to de-

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Abbreviations: GALT, gut associated lymphoid tissue; PBS, phosphate buffered saline; TCR, T cell receptor; UC, ulcerative colitis.

velop approximately two weeks after birth and rapidly mature during the first few years of life. The established appendiceal lymphoid follicles with germinal center degenerate from adolescence through the third decade, thus much smaller appendiceal lymphoid follicles are seen in older individuals [4,9]. Andre-ou et al. proposed that degeneration of appendiceal lymphoid follicles with age is associated with fibrotic change [10], whereas Miranda et al. reported no obvious correlation between fibrosis and increased age [11]. Therefore, although the appendix experiences degeneration of lymphoid tissues with aging, the mechanism of action remains unclear.

MATERIAL AND METHODS

<Patients>

Surgically resected appendix tissues from patients with colon cancer, intestinal malrotation and UC were obtained from Kurume University Hospital , Kurume Hospital and St. Mary's Hospital. This experiment has been approved by the Kurume University Ethics Committee (study number: 14253).

<Histological Analysis>

For histological analysis, specimens were fixed in 3% buffered formalin and embedded in paraffin. Multiple 4-μm sections were stained with hematoxylin and eosin. Azan staining was use to analyze fibrotic changes.

<Flow Cytometry>

Appendix lymphoid follicles were dispersed gently using 26-gauge needles and the cell suspension was passed through a nylon membrane (40μm pore size). 1.0×10^6 cells were washed with FACS buffer® (PBS containing 0.2%BSA and 0.1%sodium azide) at 4°C. After washing with FACS buffer®, cells were analyzed using FACSVerse® (BD Bioscience).

RESULTS

<Characteristics of Patients>

In total, 8 patients were included in this study. Of these, 4 patients had colon cancer, 1 patient had intestinal malrotation and 3 patients had UC. Median age was 71 years (IQR, 61-76 years). All UC patients received steroid therapy (Table 1).

<Steatotic changes in appendix>

In normal appendix, no obvious submucosal fat was recognized in cases at 5 days or 45 years of age (Figure 1. A, B). Submucosal fat became clearly recognizable in the case of a 75 year old (Figure 1. C). Submucosal fat was further extended into the mucosa in the case of an 80 year old (Figure 1. E). Therefore, steatotic changes in the appendix may be induced with aging, particularly after 70 years of age. In contrast, there was no clear association between appendiceal fibrosis and aging. In UC patients, submucosal fat was seen in the appendix of one case at 66 years of age (Figure 1. F). Therefore, UC patients may be more susceptible to steatotic change, presumably due to long-term steroid treatment. In addition, lymphoid follicular formation and mucosal structure were disrupted in the appendices of two of three UC patients at 70 and 72 years of age (Figure 1. G, H).

<Degeneration of lymphoid follicle formation>

Normal appendix is characterized by an accumulation of lymphoid follicles (Figure 1 A-C). Lymphoid follicle formation, although still immature, was recog-

TABLE 1.
Characteristics of Study Participants.

Patient	Age (years) / Gender	Cause of operation	Duration of afflicted with UC (years)	Total dose of steroid (g)
A	0/M	Intestinal duplication, Malrotation, duodenal stenosis	-	-
B	45/F	Ascending colon cancer	-	-
C	75/F	Ascending colon cancer	-	-
D	79/F	Ascending colon cancer	-	-
E	80/M	Transverse colon cancer	-	-
F	66/M	Ulcerative colitis (total colitis)	7	13.2
G	70/M	Ulcerative colitis (total colitis)	34	21
H	72/M	Ulcerative colitis (total colitis)	10	0.93

nized in the appendix at 5 days of age (Figure 1. A), and established lymphoid follicular formation was seen at 45 and 75 years of age (Figure 1. B, C). The lymphoid follicles then disappeared in the patients 79 and 80 years of age (Figure 1. D, E). Therefore, lymphoid follicles in the appendix may start to develop before 5 days of age, gradually mature, and eventually disappear with age. In the case of UC patients, one case exhibited degenerated lymphoid follicles (Figure1. F), and two cases showed no lymphoid follicles (Figure1. G, H).

<Decreased number of lymphoid cells in the appendix with aging>

Flow cytometric analysis clearly showed a lymphocyte population in the appendix at 5 days, 45 and 75 years of age (Figure2. A, B, C). In contrast, lymphoid population was hardly detectable in the cases at 79 and 80 years of age (Figure2. D, E) who showed no lymphoid follicles histologically. Interestingly, in the case at 5 days of age (Figure2. A), the forward scatter of the lymphocyte population was increased as compared to adult subjects. Therefore, it is possible that immature immune cells, as indicated by the increase in cell size, exist in the new-born appendix.

In the cytograms from appendices of UC patients, lymphocyte population was reduced in one case (Figure2. F) who exhibited degenerated lymphoid follicles histologically. As predicted, lymphocyte populations were undetectable in two cases (Figure2. G, H) who had no lymphoid follicles. These flow cytometric data support the histological findings.

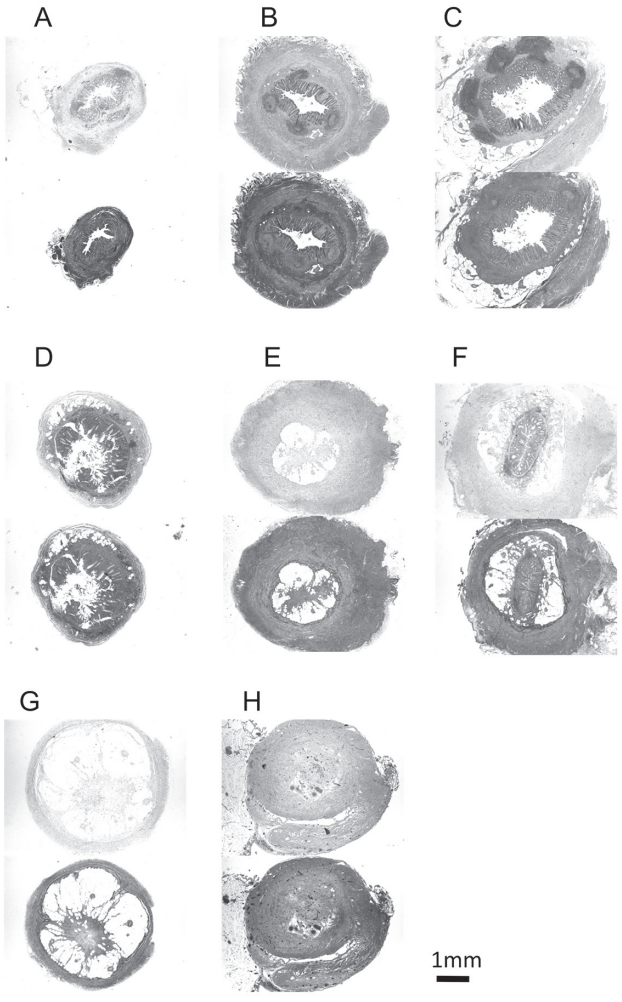

Fig. 1. Pathological specimen of appendix stained with hematoxylin eosin (upper) and azan stain (lower) is shown.
Patients with malrotation at 5 days old (A), patients with colon cancer at 45 (B), 75 (C), 79 (D) and 80 (E) years old, patients with UC at 66 (F), 70 (G) and 72 (H) ware analyzed.([A-H]×25 objective.)

DISCUSSION

Studies evaluating the age-related alteration of human appendix proposed an association of fibrosis with aging. Fibrosis was more common in older subjects (76.2%, mean age of 69.4 years) than in a younger group (4.3%, mean age of 22,5 years) [10]. In addition, submucosal fat was commonly seen with fibrosis in autopsy appendices [10]. Consistent with this report, we also found that submucosal fat was frequently seen in the appendix of older cases. However, our results show no clear association between submucosal fat and appendiceal fibrosis. Since our experiment includes only 5 cases, this discrepancy could be due to the small sample size. Alternatively, 61% of samples used in previous studies were from acute inflammation. Since acute appendicitis was excluded from our study, inflammatory factors may partly explain the difference between our findings and previous reports.

Previous studies have reported [4] that number and size of lymphoid follicles in the appendix increases after birth and starts to decrease from the third decade. Consistent with the previous findings, our study detected more lymphoid follicles in the case of a 45 year old patient than in patients 75, 79 and 80 years of old. This finding was further supported by flow cytometric analysis, which is useful in the measurement of lymphocyte populations.

In UC patients, the appendix tends to be characterized by fatty change that extends to the submucosa and mucosa areas. In addition, the lymphoid follicles of UC patients tend to disappear or be atrophied compared to healthy controls. Therefore, it is possible that chronic inflammatory insult from UC may facilitate the degenerative change in the appendix lymphoid

system. Alternatively, UC patients subjected to colectomy have a long history of steroid medication which is known to have fat deposition as an adverse effect. Therefore, it is also possible that long term treatment with steroid may facilitate the degenerative change in the appendix lymphoid follicles through local accumulation of fat.

Several groups have reported a negative association between development of UC and a prior history of appendectomy preformed before 20 years of age, suggesting a protective effect of appendectomy on UC development [7]. In contrast, the therapeutic potential of appendectomy is still controversial [12,13]. Indeed, therapeutic appendectomy after UC diagnosis has been reported to exacerbate the disease and it showed a 2.2-fold increased risk of colectomy [14].

Our data demonstrate that the appendices of UC patients have few or no lymphoid follicles. Our findings suggested that appendectomy in patients after UC diagnosis who have had early degenerative change in the appendix lymphoid follicles may be a negative factor for improvement of the clinical course of UC. However, our target patients with UC at 66,70 and 72 years of age were older than those in previous studies, and moreover, patients with UC that underwent appendectomy prior to 20 years old showed no difference in rate of colectomy compared with UC patients who did not undergo appendectomy [14]. Further investigation will be necessary to clarify the function of the appendix in the pathogenesis of UC.

CONCLUSION

Our study confirms previous findings that the appendix experiences degeneration of lymphoid tissues with age, and proposes that inflammatory insult may facilitate the degenerative process in patients with UC.

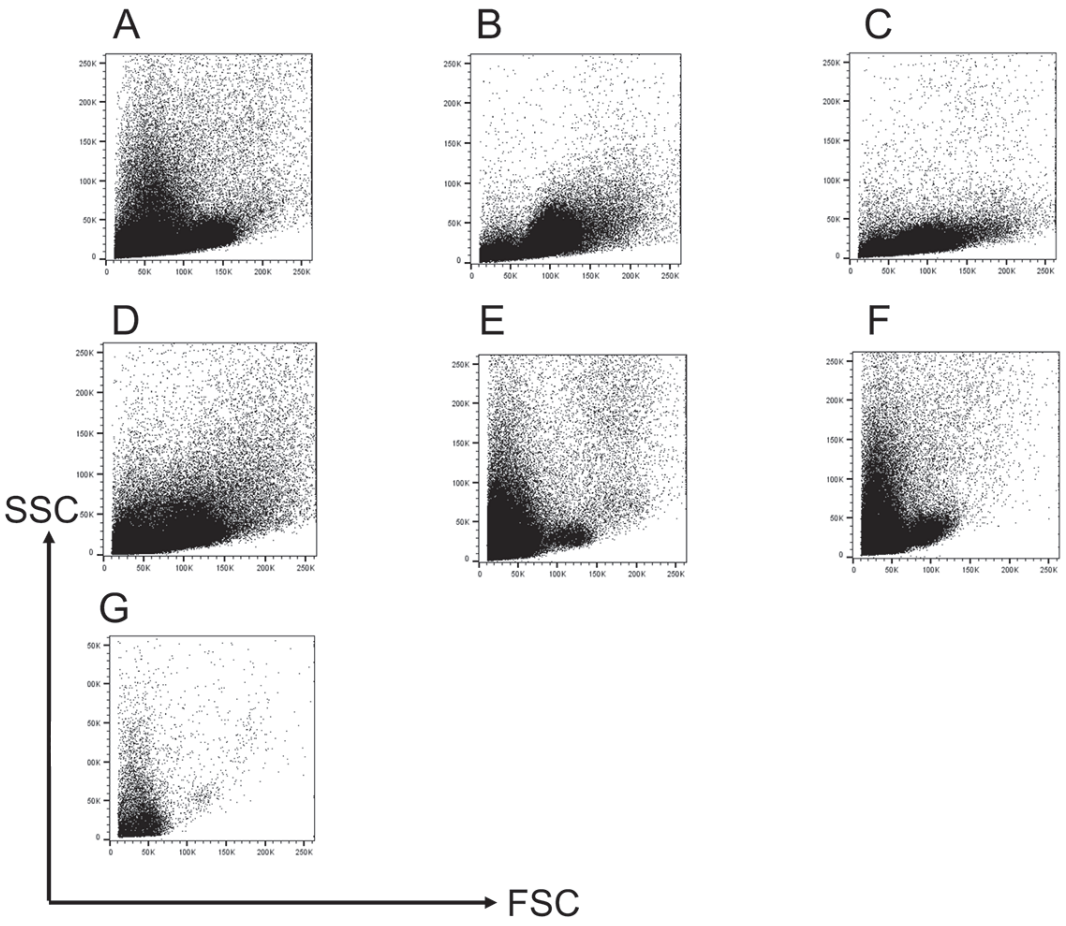

Fig. 2. Cytogram in flow cytometric analysis is shown. (A) – (H) are same patients at Figure 1. FSC, forward scatter; SSC, side scatter.

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