

Endoscopic and Histological Features of the Large Intestine in Patients with Atopic Dermatitis

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Summary Although atopic dermatitis is known to be closely associated with food antigens, the actual changes in the gastrointestinal tract have not been clarified. The aim of this study was to investigate the macroscopic and histological features of the large intestine in patients with atopic dermatitis. We studied 15 outpatients who had generalized atopic dermatitis. Eight non-dermatitis subjects of a similar age without inflammatory bowel disease were also enrolled as controls. Total colonoscopy, pathological evaluation of biopsy specimens, and detection of *Candida albicans* were performed in all subjects. Four patients were re-examined after 6 months of treatment with an antifungal drug. Among the 15 patients with atopic dermatitis, 4 patients had melanosis coli. On pathological examinations, prominent infiltration of eosinophils and fragmentation of granulocyte nuclei were observed. There were no changes after an antifungal therapy. In the patients with melanosis coli, lipofuscin deposits were observed in the lamina propria. *Candida albicans* was not detected in any of the subjects. In conclusion, patients with atopic dermatitis may have a predisposition to develop chronic inflammation of the large intestine.

Key Words: atopic dermatitis, large intestine, melanosis coli

Introduction

Recently, adult atopic dermatitis has increased rapidly in Japan. Although various factors contribute to the development of atopic dermatitis, food allergy has attracted attention and the contribution of food antigens to disease is considered to be important [1–5]. Treatment of atopic dermatitis by removing certain food antigens from the diet has shown some clinical success [6–10].

On the other hand, since Crook *et al.* proposed the “Yeast Connection” [11, 12], many reports have suggested that mycosis may be associated with atopic dermatitis [13–19] and some previous studies have demonstrated that administration of antifungal agents is effective for improving this type of dermatitis [20–22]. However, it is still unclear whether or not intestinal candidiasis plays a role in atopic dermatitis.

Although an association between the digestive tract and atopic dermatitis has long been assumed, there have been few direct studies of the digestive tract in patients with this condition [23]. In order to determinate the state of the digestive tract in patients with atopic dermatitis, and whether mycosis intestinalis plays a role in this condition, a direct investigation of the digestive tract in outpatients without

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specific abdominal symptoms is considered to be necessary.

We examined the large intestine in atopic dermatitis patients without specific abdominal symptom to clarify the gross and histological features of the large bowel in this condition. The possible contribution of intestinal candidiasis to atopic dermatitis was also studied.

Materials and Methods

Patients

Atopic dermatitis patients attending Aichi Medical University Hospital or Hoshigaoka Dermatology Clinic over the past several years were enrolled in this study. Their average age was 26.1 ± 2.3 years and the male to female ratio was 13:2. All patients were in a good general condition, but had eruptions with areas of lichenification and exudation, skin pigmentation, and epilation distributed over the entire body. The bowel habits of all patients were normal. They had no specific abdominal symptoms and did not abuse laxatives that contained senna, aloe, or other ingredients. All patients were being treated with anti-allergy agents and occasionally used topical preparations containing corticosteroids. They had not taken oral steroids for at least 3 months before enrollment. As a control group, 2 healthy adult volunteers and 6 subjects with no atopic dermatitis who complained of abdominal symptoms such as constipation but did not have inflammatory bowel disease on colonoscopy also participated in this study. Their average age was 23.4 ± 2 years and the male to female ratio was 7:1. Endoscopic examination of the large intestine was performed at endoscopy center of Nagoya University Hospital after obtaining a written informed consent. An antifungal drug (*itraconazole* at 100 mg/day, Janssen Pharmaceutical K.K., Tokyo, Japan) was administered to 4 dermatitis patients for 6 months, and endoscopic examination was performed again at the time when skin eruptions had improved.

Colonoscopy and Preparation of Biopsy Specimens

The subjects fasted with free access to water from the morning of examination. They each drank 2 L of *Niflec* (a polyethylene glycol electrolyte solution, Ajinomoto Pharma Co. Ltd., Tokyo, Japan) over 2 hours from 9:00 a.m., and examinations were started at 1:30 p.m.. As premedication, 20 mg of scopolamine butylbromide was administered intramuscularly. The colonoscope was inserted to as far as the cecum and observation was performed with drawing it. Biopsy specimens were taken randomly from the distal descending colon, when localized lesion was not observed. One part of each specimen was fixed in 10% buffered formalin and embedded in paraffin. The other part was fixed in 4% paraformaldehyde for 6 h, immersed in 20% sucrose/PBS solution for 30 min., embedded in OCT compound, and frozen at -80°C .

Histological Evaluation

Eosinophil infiltration was classified into 4 grades from 0 to 3+ (0: almost none, 1+: slight, 2+: moderate, 3+: severe) by examination of hematoxylin-eosin stained 3- μm sections of paraffin-embedded specimens. Classification was done by pathologists without access to any clinical information, as previously reported [24]. Fragmentation of granulocyte nuclei and lipofuscin deposits were also evaluated in the same manner.

Detection of *Candida Albicans*

Detection of *Candida albicans* was performed by immunohistochemical staining of 5- μm frozen sections using the streptavidin-biotin method. The primary antibody was anti-*Candida albicans* mouse monoclonal antibody (Chemicon International, Inc., Temecula, CA). In addition, DNA was extracted from 10- μm frozen sections using DNAzol (Molecular Research Center Inc., Cincinnati, OH) and PCR with a specific primer set for the secretory aspartate proteinase of *Candida albicans* was performed under the conditions shown in Table 1.

Statistical Analysis

Values are expressed as the mean \pm S.E. The Wilcoxon's rank test was used for assessment of differences in pathological features and $p < 0.05$ was considered significant.

Results

Endoscopic Findings

Total colonoscopy could be performed without any problems in all cases. In the patients with atopic dermatitis, the long sigmoid colon is commonly coiled inside the pelvis.

Macroscopically, no abnormal findings were observed in the control group, whereas marked melanosis coli were found in 4 of the 15 patients with atopic dermatitis (Fig. 1). After treatment with an antifungal drug for 6 months, there was no change in the extent of melanosis coli (Fig. 1), although the skin eruptions showed improvement.

Histological Findings

Table 2 summarized the results of histological examination. Eosinophil infiltration and granulocyte nuclear fragments

Table 1. Condition for PCR to detect the secretory aspartate proteinase of *Candida albicans*.

Forward	5'-TTGCCGATATCACCAAGACTTCTATTCCCTC-3'		
Reverse	5'-TGAGAGAATTCAAAGTGATTCTTAATTCTC-3'		
	Denaturation	94°C	60 sec.
	Annealing	58°C	40 sec.
	Extension	72°C	45 sec.
	40 cycles		

A 273-bp product was obtained by this PCR reaction.

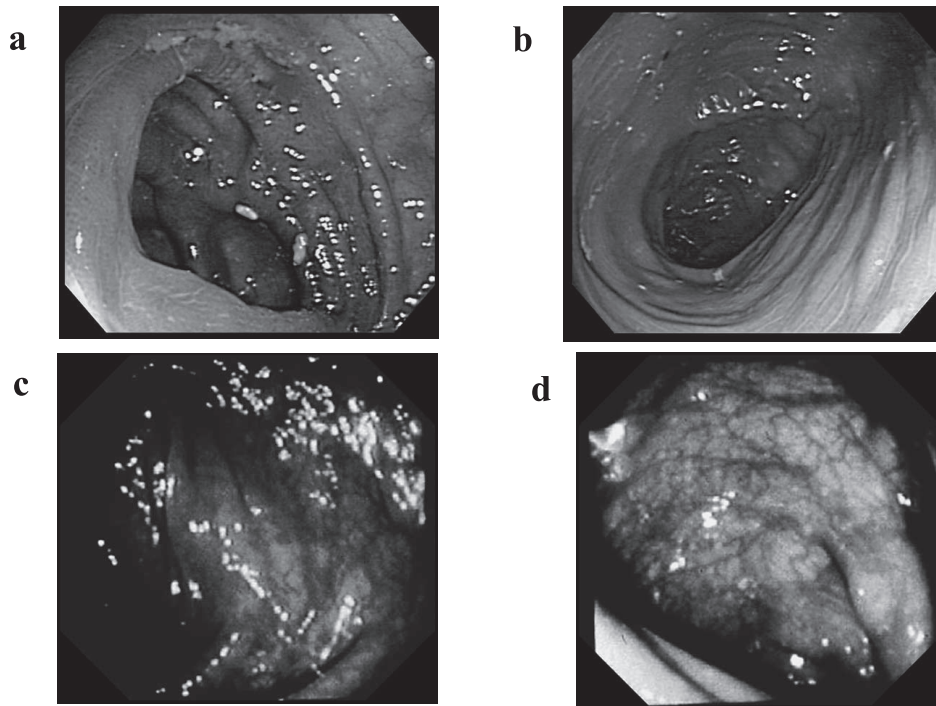


Fig. 1. Endoscopic features of melanosis coli. The ascending colon (a) and descending colon (b) of patient no. 8, and the transverse colon of patient no. 13 before (c) and after (d) treatment with itraconazole.

Table 2. Histological findings of the large intestine in both groups.

Subject no.	Age	Sex	Peripheral Eosinophil count (/ μ L)	Total IgE level (IU/mL)	Eosinophilic infiltrations	Nuclear fragments	Lipofuscin deposits
1	28	M	984	23000	3+	2+	-
2*	38	M	448	23000	3+	2+	2+
3	22	M	610	15000	3+	2+	-
4	24	M	279	13000	3+	2+	-
5	20	M	972	13000	3+	2+	+
6*	20	M	760	10270	2+	+	-
7	33	M	324	10000	+	-	-
8*	23	M	451	8200	+	+	2+
9	23	M	682	6700	2+	+	-
10	18	M	702	6610	+	-	-
11*	26	M	532	5300	2+	+	-
12	19	M	592	4400	2+	+	-
13	37	F	913	4200	2+	+	+
14	47	F	310	2900	2+	+	-
15	14	M	798	1500	2+	2+	-
C1	26	M			+	+	-
C2	21	M			-	-	-
C3	20	M			+	+	-
C4	18	M			+	+	-
C5	17	M			+	-	-
C6	22	F			-	-	-
V1	30	M			+	-	-
V2	33	M			+	-	-

1–15: Patients with atopic dermatitis, C1–6: Controls, V1,2: Volunteers

*: Repeat colonoscopy was performed after 6 months of treatment with itraconazole.

∴: Cases with melanosis coli.

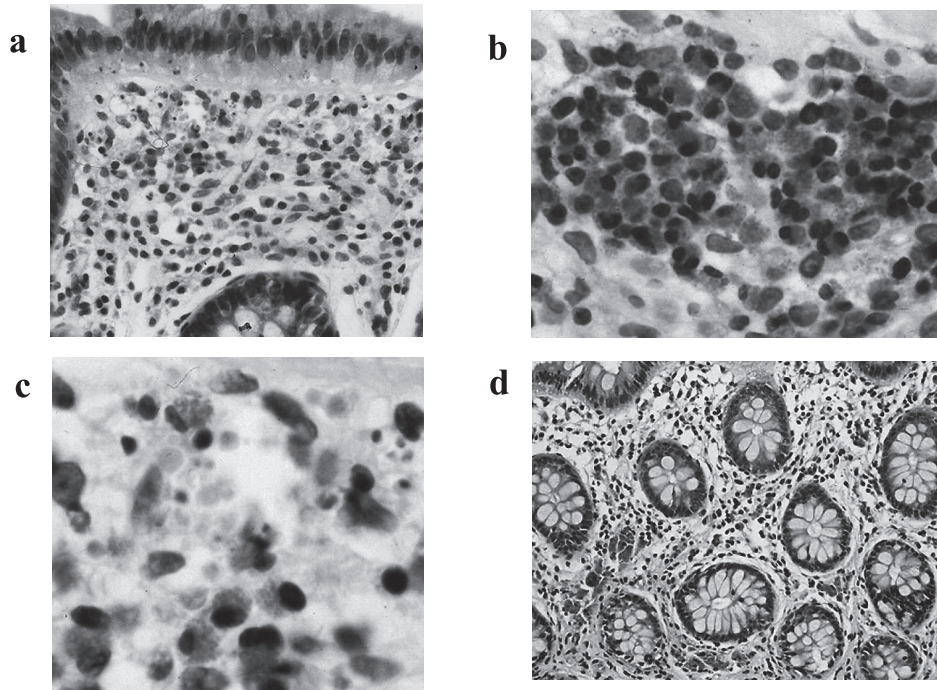


Fig. 2. Histological findings of biopsy specimens from the descending colon in patients with atopic dermatitis. Numerous eosinophil are infiltrating into the lamina propria (a). A cluster of eosinophil (b) and fragments of granulocyte nuclei are observed (c). In association with melanosis coli, lipofuscin deposits are seen (d).

were significantly more common in the atopic dermatitis group than the control group (both $p < 0.05$, Figs. 2 and 3). There was no relationship between the peripheral blood eosinophil count and the extent of eosinophil infiltration into the colonic mucosa. In the patients with melanosis coli, lipofuscin deposits were also observed in the lamina propria (Fig. 3). The total IgE level showed no association with any of these histological findings. The histological changes did not improve in any of the 4 patient cases after treatment with an antifungal drug for 6 months, although skin eruptions were alleviated.

Candida albicans

On histochemical and genetic analysis, *Candida albicans* was not detected in any of the subjects (Fig. 4). The same result was also obtained after antifungal therapy.

Discussion

This is the first study to directly investigate the characteristics of large intestine in patients with atopic dermatitis. Macroscopically, there were no abnormal findings in 11 of over 15 patients, whereas prominent melanosis coli were seen in the other 4 patients (26.7%). This prevalence was far higher than that reported by Watanabe *et al.* [25] (57 cases of melanosis coli in 6,293 persons undergoing routine proctosigmoidoscopy). It is generally recognized that melanosis

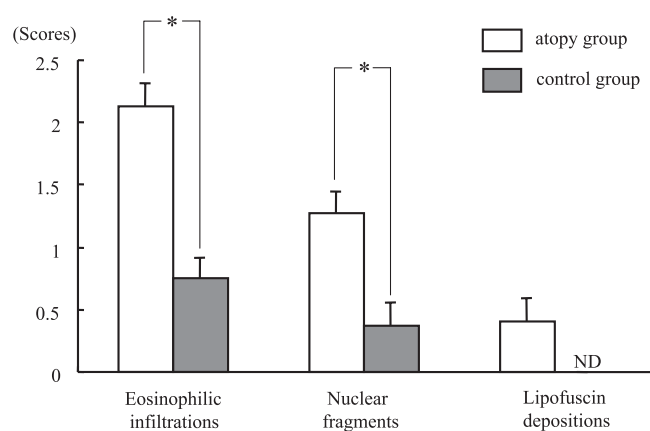


Fig. 3. Histological scores. In the atopic dermatitis group, the scores for both eosinophil infiltration and fragmentation of granulocyte nuclei were significantly higher than in the control group. Values are shown as the mean \pm SE. * $p < 0.05$.

coli are observed in elderly persons who have a history of chronic laxative use [26–28]. However, our patients were so young, had no abdominal symptoms, and no history of laxative abuse.

The melanosis reflects the lipofuscin depositions in mucosa pathologically, and these lipofuscin depositions induced by the binding of lipid hydroperoxides to proteins is understandable

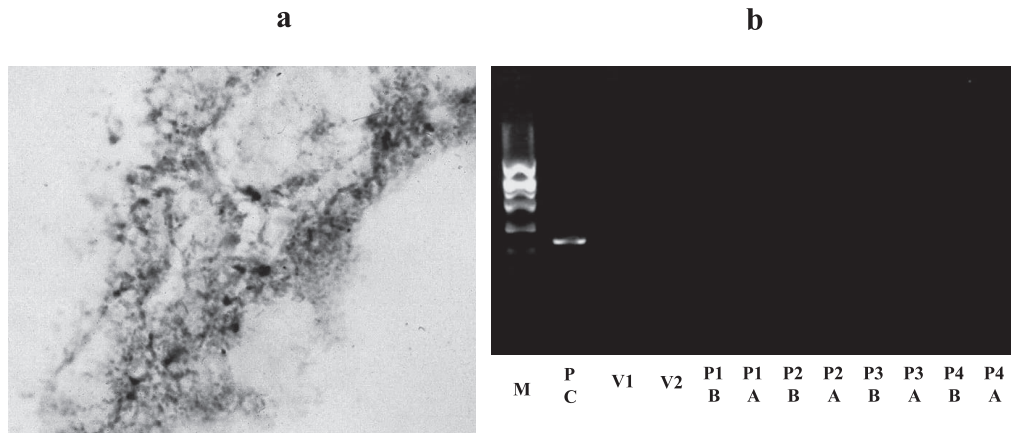


Fig. 4. Detection of *Candida albicans*. Using both immunohistochemistry (a) and the PCR (b), *Candida albicans* was not detected in the large intestine of any subjects.

M: Marker, PC: Positive control, Vx: Volunteers, PxB and A: Patients with atopic dermatitis before (B) and after (A) treatment with itraconazol.

to be findings with aging [29–32]. On the other hand, melanosis is also considered to be a sign of chronic inflammation [27, 33–35]. Indeed, melanosis coli is reported to be associated with inflammatory bowel diseases [36]. Because pathological findings, such as eosinophil infiltration and fragmentation of granulocyte nuclei, were seen in our patients with atopic dermatitis, the melanosis coli observed in this study may be caused by chronic inflammation. Recently, Niwa *et al.* have been reported that atopic dermatitis is part of the spectrum of diseases related to impaired epithelial barriers and may be associated with ulcerative colitis [37]. Further, it was reported that hypoproteinemia as a complication of severe atopic dermatitis was caused by loss of protein through the gastrointestinal tract [38]. Thus, although neither erosions nor ulceration were observed in our study, latent chronic inflammation may be present in the large intestine of atopic dermatitis patients.

However, this inflammation may not be a cause of atopic dermatitis because there was no improvement of melanosis coli or inflammatory cell infiltration over 4 patients, whose dermatitis improved after 6 months of treatment with an antifungal drug. This finding also suggested that there was little association between intestinal candidiasis and chronic inflammation of the large intestine. In the present study, we attempted to detect *Candida albicans* in the colonic mucosa using PCR and immunohistochemistry of biopsy specimens. Although these methods are very sensitive, it is impossible to assess the entire large intestine, so false-negative results may be obtained. It might be suggested that we should have used fecal culture, but we employed these methods in order to avoid the risk of contamination. Our study revealed no *Candida albicans* in all the 15 patients. In 4 patients who underwent treatment with an antifungal drug, *Candida albicans* was also not detected after treatment.

These results provide further evidence of the lack of an association between *Candida albicans* and endoscopic and histological changes of the colon in patients with atopic dermatitis.

Conclusions

The present prospective study suggested that patients with atopic dermatitis might have an latent chronic inflammation in the large intestine, although this was not a strict case-control study. In addition, *Candida albicans* seems to have little influence on chronic inflammation of the large intestine.

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