Original Article

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Peyer's Patches in the Terminal Ileum in Ulcerative Colitis: Magnifying Endoscopic Findings

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Summary Peyer's patches (PPs), a major component of the gut-associated lymphoid tissue, serve as important antigen entry sites in mucosal immunity. PPs may play a role in the extension of ulcerative colitis (UC) into the terminal ileum. We sought to clarify the magnified endoscopic findings of the PPs in the terminal ileum of UC patients. Eighteen UC patients underwent magnifying chromoendoscopy before initial treatment to evaluate the follicle-associated epithelium (FAE) on the PPs domes and the surrounding villi. In 8 UC patients, as in healthy controls, the PPs' domes were slightly elevated, covered with the regular FAE lining, and surrounded by dense and bulky villi; however, in 10 UC patients, the PPs' domes were irregular, and the surrounding villi were sparse and atrophic. These abnormal findings within the PPs were associated with minimal mucosal lesions but not with backwash ileitis; both electron microscopy and magnifying endoscopy confirmed that these lesions were reversible following remission with prednisolone-mesalazine therapy. Similar to Crohn's disease patients, UC patients commonly had abnormalities in the FAE on PPs' domes and the surrounding villi on magnifying endoscopy.

Key Words**:** Peyer's patches, ulcerative colitis, magnifying endoscopy, follicle associated epithelium, M cells

Introduction

Gut-associated lymphoid tissue (GALT) is the largest lymphoid organ in the body [*1*]. GALT has to recognize and allow the transfer of beneficial antigens while concurrently dealing with and successfully removing putative and overtly harmful antigens. This distinctive biological feature of GALT is crucial to good health, whereas the deregulation or dysfunction of GALT is thought to predispose to inflammatory bowel disease, ulcerative colitis (UC) and Crohn's disease (CD) [*1–3*].

Peyer's patches (PPs) are central to mucosal immunity as they play a major role in GALT; they modulate intestinal immune and inflammatory responses or tolerance [*1, 3*]. PPs are widely distributed along the small intestine but their highest density is in the human ileum [*4*]. PPs are concentrated in the distal 25 cm of the ileum [*4*]; there they serve as

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important antigen entry sites for the immune system. PPs consist of 10–1000 individual follicles organized into discrete lymphoid structures that are overlaid by follicle-associated epithelium (FAE) [4, 5].

In patients with CD, involvement of the terminal ileum is most common, and the initial mucosal lesions of CD often appear over PPs [2]. More recently, using magnifying chromoendoscopy, we identified aphthous erosions in the FAE on the domes of PPs [6]. In addition, most CD patients examined had irregularly even PPs domes surrounded by sparse villi. Of note, non-caseous epithelioid granuloma were frequently identified in the biopsies taken from PPs [6]; this supports the concept that CD lesions may originate from PPs [3, 7].

However, scant attention has been paid to the association of PPs with the pathogenesis of UC. In this regard, there is no information dealing with detailed macroscopic observations of the terminal ileum of UC patients with special reference to PPs. UC is a chronic inflammatory condition that invariably involves the rectum and over time often extends proximally in a continuous manner [2]. UC does not typically involve other areas of the gastrointestinal tract, but further extension of the inflammatory process into the terminal ileum is common [8, 9]. It is possible that PPs play a role in the development of the ileal lesions seen in UC patients. In the present study, the magnified endoscopic findings of PPs in the terminal ileum of UC patients were clarified.

Patients and Methods

Between 1998 and 2005, 18 UC patients without any prior treatment were enrolled. The diagnosis of UC was made on the basis of the endoscopic, radiological, histological, and clinical criteria provided by the WHO Council for International Organizations of Medical Sciences and the International Organization for the Study of Inflammatory Bowel Disease [10, 11]. Patients with Crohn's disease, indeterminate colitis, and autoimmune diseases, such as RA, multiple sclerosis, and systemic lupus erythematosus, were excluded from the study. The patients were classified into subgroups according to the extent of their lesions (proctitis, left-sided colitis, or pancolitis), disease severity (mild, moderate, or severe), and activity (active or inactive) [12].

After usual premedication, colonoscopic examination was performed using a magnifying videocolonoscope with an image processor (Olympus CF-240ZI). After routine conventional observation, chromoendoscopic observation was performed using 0.1% indigo carmine, which was sprayed directly onto the mucosal surface of the terminal ileum to identify the PPs. The FAE on the PPs' domes and the surrounding villi was examined by magnifying endoscopy. According to the classification that we previously

proposed, the macroscopic appearance of PPs was divided into two categories: type E, consisting of a nodular or convoluted elevation pattern; and type F, consisting of a flat pattern [6]. During endoscopy, biopsy specimens were taken from the PPs' domes; one of the specimens was fixed in formalin, embedded in paraffin wax, and stained with hematoxylin and eosin for histopathological examination.

The following histologic features were evaluated in accordance with previous report [9]: 1) presence, type, and degree of ulceration; 2) severity and extent of active (neutrophilic) inflammation. Inflammation was noted as to whether it was limited to the lamina propria, infiltrating crypts (cryptitis), forming crypt abscesses or whether it was associated with ulceration. 3) Degree of mononuclear inflammation in lamina propria, graded as normal, or increased. 4) Extent of villous atrophy was graded as none, subtotal, or total.

In certain cases, the biopsy sample was prepared for electron microscopy; each specimen was rinsed with physiological saline solution, fixed in 2.5% glutaraldehyde at 4°C, and then fixed in phosphate buffered 1% osmium peroxide (pH 7.4) and in 1% tannic acid overnight, followed by 1% osmium tetroxide and dehydration through a graded ethanol series. After drying in a critical point dryer, the specimens were coated with platinum palladium and observed with a Hitachi S-570 scanning electron microscope [6, 13].

The Statistical significance of differences in patients' characteristics and clinicopathological features was determined using Fisher's exact test, the χ^2 test, the Mann-Whitney U test, or Student's t test, as appropriate. p values less than 0.05 were considered statistically significant.

All examinations were conducted according to Good Clinical Practice and the Declaration of Helsinki, and were approved by the university ethics committee. All samples were obtained after written informed consent was obtained from the patients prior to their inclusion in the study.

Results

The characteristics of the 18 UC patients are summarized in Table 1. There were 11 men; the patients' mean age was 36 years (range, 16 to 72 years). Twelve patients had pancolitis, two had left-sided colitis, and four had proctitis. All of the patients but one had active UC. On chromoendoscopy, 9 UC patients had type E PPs (Fig. 1A), and 9 had type F PPs (Fig. 1B). The type E PPs were associated with definite lymphoid follicles, as well as abundant lymphoid hyperplasia (Fig. 1C), whereas the type F PPs were associated with aggregated lymphocytes without distinct follicle formation (Fig. 1D). During the same time period, 140 subjects without any gastrointestinal disorders underwent chromocolonoscopy to evaluate PPs in the terminal ileum (Table 2). There was no difference in the age distribu-

Table 1. Clinical characteristics, endoscopic appearance of Peyer's patches (PPs), and magnified chromoendoscopic findings of the PPs in 18 patients with ulcerative colitis (UC)

No	Age years	Gender	Extent of UC	Disease severity	Disease activity	Appendix	PPs' type*	Ileal lesions	Medications	Treatment outcomes	Before Treatment Abnormal features#	After treatment Abnormal features#
1	16	Male	pancolitis	severe	active	uninvolved	E	none	prednisolone + mesalazine	remission	absent	absent
2	18	Female	left-sided	moderate	active	uninvolved	E	none	prednisolone enema	remission	absent	not done
3	19	Male	left-sided	moderate	active	uninvolved	E	none	prednisolone + mesalazine	remission	present	improved
4	23	Female	proctitis	none	inactive	uninvolved	E	none	not done	—	absent	not done
5	23	Male	proctitis	mild	active	uninvolved	E	none	prednisolone enema	active	absent	not done
6	26	Male	pancolitis	moderate	active	uninvolved	E	none	prednisolone + mesalazine	remission	present	improved
7	27	Male	pancolitis	mild	active	involved	F	none	prednisolone + mesalazine	remission	absent	absent
8	27	Male	pancolitis	moderate	active	uninvolved	E	none	prednisolone + mesalazine	remission	absent	absent
9	31	Male	proctitis	mild	active	uninvolved	E	reddishness	mesalazine	remission	present	unchanged
10	32	Male	pancolitis	mild	active	uninvolved	F	erosions	mesalazine	remission	present	unchanged
11	33	Male	pancolitis	moderate	active	uninvolved	E	erosions	prednisolone + mesalazine	remission	present	improved
12	39	Female	pancolitis	moderate	active	involved	F	none	prednisolone + mesalazine	remission	present	improved
13	45	Male	pancolitis	moderate	active	uninvolved	F	erosions	prednisolone + mesalazine	remission	present	improved
14	50	Female	pancolitis	mild	active	uninvolved	F	reddishness	mesalazine	remission	present	unchanged
15	52	Female	pancolitis	moderate	active	uninvolved	F	none	prednisolone + mesalazine	remission	absent	absent
16	54	Female	proctitis	moderate	active	uninvolved	F	erosions	prednisolone enema	active	present	not done
17	66	Female	pancolitis	moderate	active	uninvolved	F	none	prednisolone + mesalazine	remission	present	improved
18	72	Male	pancolitis	mild	active	uninvolved	F	none	prednisolone + mesalazine	remission	absent	absent

*: The macroscopic appearance of the PPs on chromoendoscopy with indigocarmine was classified into two categories: type E (nodular or convoluted elevation pattern); and type F (flat pattern). #: Abnormal features included unevenly flat domes of PPs that were irregular in size and shape and surrounded by atrophic villi on magnified chromoendoscopy with indigocarmine.

tion of the type E and type F PPs between the 140 controls and the 18 UC patients; in both groups, type E PPs were exclusively observed in individuals younger than 30 years, while type F PPs were predominantly observed in individuals older than 30 years. Ileal lesions were present within the PPs in 6 UC patients, 4 of whom had pancolitis and 2 of whom had proctitis; of the 6 UC patients, disease activity was mild in 3 and moderate in 3.

On magnifying chromoscopy, the PPs' domes rose into a little mound and were surrounded by dense villi (Fig. 2A) in 8 UC patients, whereas 10 UC patients had abnormal domes that were unevenly flat with irregular sizes and shapes and surrounded by atrophic villi that were sparse (Fig. 2B). Six of these ten UC patients had ileal lesions. The endoscopic findings of these 6 UC patients were mild, with few erosions and redness within the PPs (Fig. 3A). No patients had distinct backwash ileitis. Although the appendix was involved in 2 of the UC patients, the macroscopic appearance was mild, with redness and edema, and there were no associated ileal lesions.

Post-treatment magnifying endoscopy done 3 to 10 months (mean 8 months) later in 13 UC patients after remission with oral treatment (prednisolone plus mesalazine in 10 patients or mesalazine alone in 3 patients). In the 3 patients treated with mesalazine alone, there was no change in the magnified endoscopic appearance of PPs with treatment (Fig. 4A and B). On the other hand, in 6 of 10 of the patients treated with

dual therapy, the irregularity of the PPs' domes (Fig. 2B), had improved, and they were surrounded by more dense and bulky villi (Fig. 4C); however, the endoscopic category of the PPs remained unchanged after treatment.

Histologically, no ulcer was identified in the biopsies taken from PPs. Active inflammation in association with cryptitis, abscess or ulceration was not seen in any cases. Increased mononuclear cell infiltration was identified in 7 cases, and was associated with the presence of villous atrophy. In fact, 6 of the 7 cases with the increased mononuclear cell infiltration exhibited villous atrophy, while none (0/11) lacking mononuclear cell infiltration had villous atrophy ($p<0.01$). Among the 7 patients with the increased mononuclear infiltration, biopsies under endoscopy were performed in 6 ones, who had achieved remission with prednisolne plus mesalazine or mesalazine monotherapy. Mesalazine alone ($n=2$) did not alter the histological findings including the increased mononuclear cell infiltration and villous atrophy. On the other hand, in 4 patients who had achieved remission with the dual therapy, the histological abnormalities were disappeared in 2, whereas the increased mononuclear cell infiltration and villous atrophy were still present in 2 and 1, respectively.

Electron microscopic observation using the biopsy specimens taken from the PPs showed the presence of M cells in the FAE (Fig. 5A) on the PPs' domes in every case that was examined. Of note, the surrounding villi were found to be
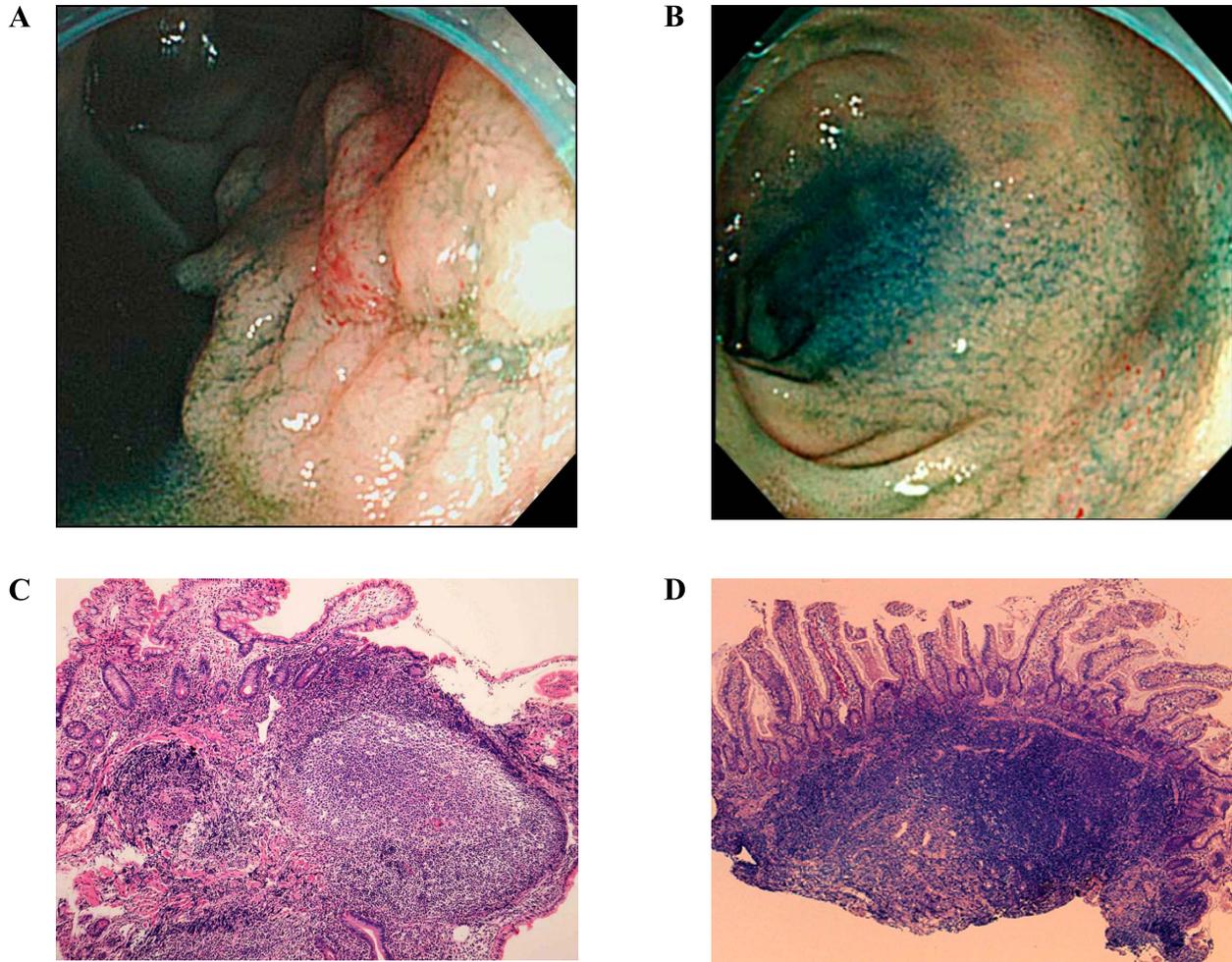
Fig. 1.   The macroscopic appearance of Peyer's patches on chromoendoscopy with indigocarmine was classified into two categories: type E (nodular or convolute elevation pattern, **A**) and type F (flat pattern, **B**). The type E PPs are associated with definite lymphoid follicles and abundant lymphoid hyperplasia (**C**, microscopic magnification ×40), whereas the type F PPs show aggregated lymphocytes without distinct follicle formation (**D**, microscopic magnification ×40).

Table 2.   Age distributions of type E (nodular or convolute elevation pattern) and type F (flat pattern) Peyer's patches (PPs) in subjects without gastrointestinal disorders

Age (years)	Number of subjects		Total numbers
	Type E PPs	Type F PPs	
0–19	3	0	3
20–29	6	3	9
30–39	2	11	13
40–49	2	25	27
50–59	2	29	31
60–69	0	36	36
70–79	0	21	21
Total numbers	15	125	140

longer and thicker after treatment with prednisolone plus mesalazine (Fig. 5B and C).

Discussion

This is the first study to document the detailed macroscopic appearance of PPs in the terminal ileum of UC patients using magnified endoscopy. Prior to initial treatment, in 8 of the 18 UC patients, the PPs' domes were slightly elevated, covered with the regular FAE lining, and surrounded by dense and bulky villi, as previously observed in healthy controls [6]. However, in 10 UC patients, the PPs' domes were irregular and surrounded by atrophic villi that were sparse. Similar features have been observed in most CD patients [6, 13]. The PPs' domes irregularity improved in more than half of the patients who achieved remission with prednisolone plus mesalazine therapy, which has also
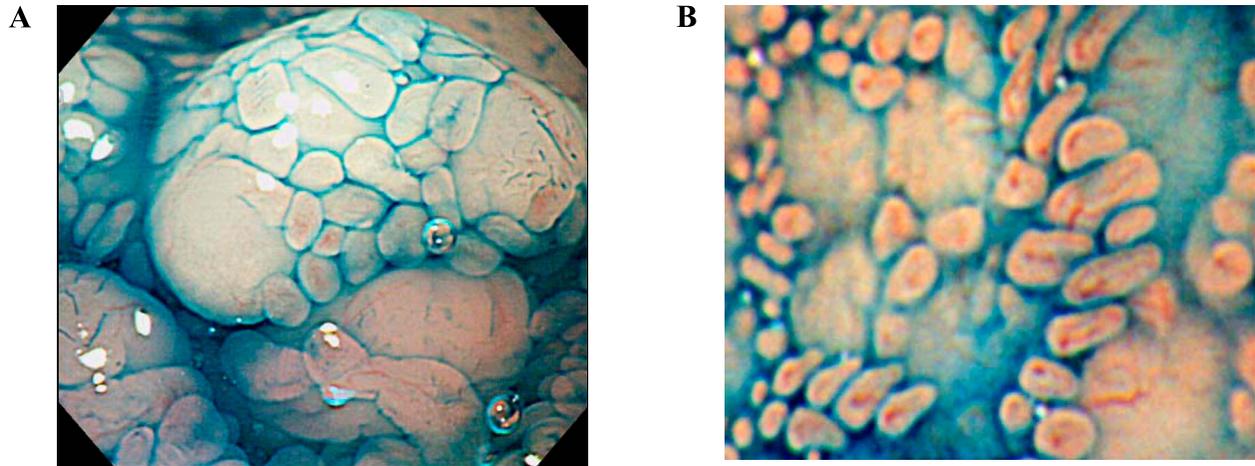
A

B

Fig. 2.   Magnifying chromoscopy with indigocarmine shows that the domes of the Peyer's patches form a little mound surrounded by dense villi (**A**). **B** shows uneven flatness of the domes of Peyer's patches, which are irregular in size and shape and surrounded by atrophic villi.
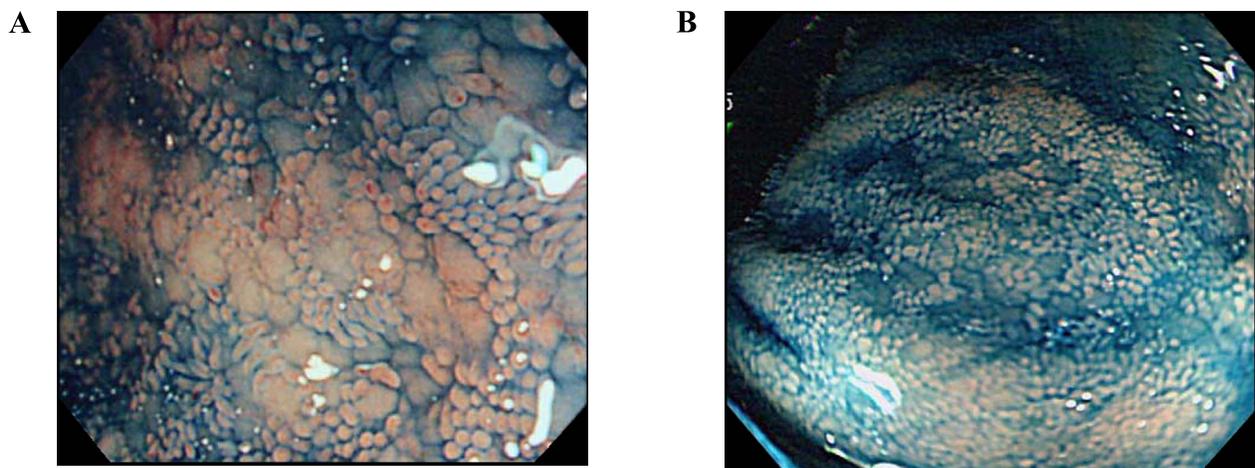
A

B

Fig. 3.   **A** shows minimal mucosal change (erosive redness) within the Peyer's patches (endoscopic magnification ×80). Following disease remission with oral prednisolone plus mesalazine therapy, the ileal lesions are no longer seen (**B**, endoscopic magnification ×80).

been seen with treatment in CD patients [6]. The scattered atrophic villi seen prior to treatment became more bulky and were more densely distributed around PPs' domes after successful treatment with prednisolone plus mesalazine, which was confirmed by not only magnified endoscopic but also electron microscopic examination. Although it is of clinical usefulness to observe the PPs with magnified endoscope before and after treatment for inflammatory bowel diseases, endoscopists require training to gain early proficiency in this technique and to overcome flat learning curves.

Mild ileal changes, such as a few erosions and redness within the PPs, were seen in 33% (6/18) of UC patients

who had chromoendoscopy in the terminal ileum. Of note, all 6 of these patients had abnormal features within the PPs on magnified endoscopy; this suggests an association between PPs and the occurrence of ileal lesions in UC patients. Also, there was a significant association of mononuclear cell infiltration with villous atrophy of PPs in UC patients, suggesting that Immune and inflammatory processes may cause villous cell components, linking to villous atrophy of the terminal ileum, in particular PPs, as seen in other sites of the gastrointestinal tract. Nevertheless, during the study period, no association with the development of overt ileitis was noted. Most patients with UC-associated ileitis have severe/fulminant activity that involves the entire
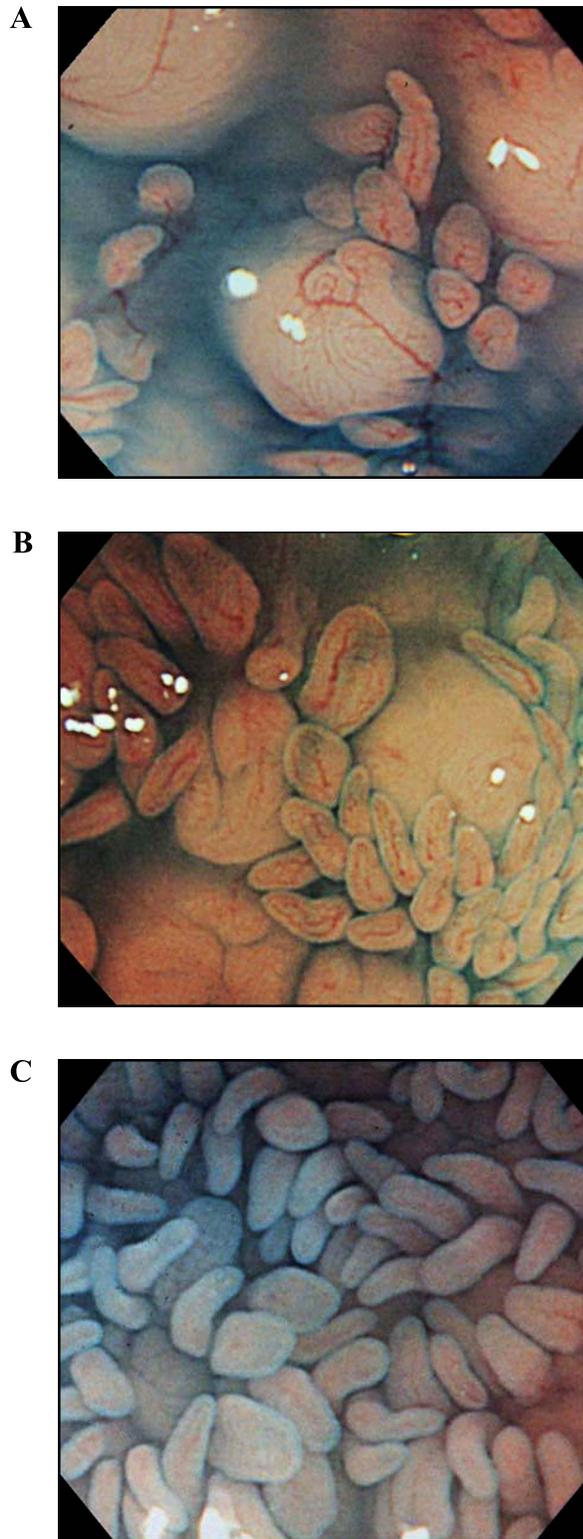
A

B

C

Fig. 4. Treatment with mesalazine alone failed to improve the irregularity in the domes of the Peyer's patches and the surrounding villous atrophy despite achieving disease remission (before and after therapy, **A** and **B**, respectively, endoscopic magnification ×80). On the other hand, the abnormal features seen in **B** are almost completely gone after remission with oral prednisolone plus mesalazine therapy; the domes of the Peyer's patches can be seen to be covered with regular lining follicle-associated epithelium and surrounded by denser and bulky villi (**C**, endoscopic magnification ×80).

colon [*8, 9*]. It is commonly thought that severe, continuous colonic disease may lead to incompetence of the inflamed ileocecal valve, which may result in retrograde flow of colonic contents into the distal ileum, resulting in inflammation [*8, 9*]. Indeed, among our patients, minimal lesions within PPs were frequently related to pancolitis. However, some patients with proctitis had ileal changes without cecal involvement. In addition, disease severity in UC patients with ileal lesions was mild to moderate. Although in many reported cases the pattern of UC-associated ileitis is consistent with a backwash etiology [*8, 9*], our data suggest that other pathogenetic factors should be considered in the pathogenesis of ileal involvement by UC.

In a long-term follow-up study of 29 patients who had isolated mucosal erosions in the terminal ileum, Goldstein found that 29% of patients developed ileocolonic CD, and that 14% were taking nonsteroidal anti-inflammatory drugs [*14*]. The etiology of ileitis was undetermined in the remaining 16 patients. In the present study, none of the UC patients had regularly taken nonsteroidal anti-inflammatory drugs, and there was no histopathological evidence of noncaseous epithelioid granuloma, which is frequently seen in biopsies taken from the PPs of CD patients [*6*]. Treatment with mesalazine alone failed to improve the irregularity of the PPs' domes and the surrounding villous atrophy despite the colonic disease achieving remission. Clearly, larger prospective studies are warranted to determine the etiology of the abnormalities within the PPs in UC patients and to clarify their implications for long-term clinical outcomes, including the occurrence of backwash ileitis.

It is unknown whether variations in the macroscopic appearance of PPs have any relationship to patients' susceptibility to disease or to the presence of disease. The age distribution of the two endoscopic categories did not differ between UC patients and controls. Irrespective of the presence of disease, type E was more common in patients younger than 30 years of age, while type F was more common in patients older than 30 years of age. In line with this, ileal lesions were also observed only in patients older than 30 years of age. This may be consistent with the findings of Cornes that the numbers of PPs peaks at ages 15–25 years and then declines with age [*15*].

The FAE located over the PPs' domes that were surrounded by scattered, atrophic villi is likely to be more
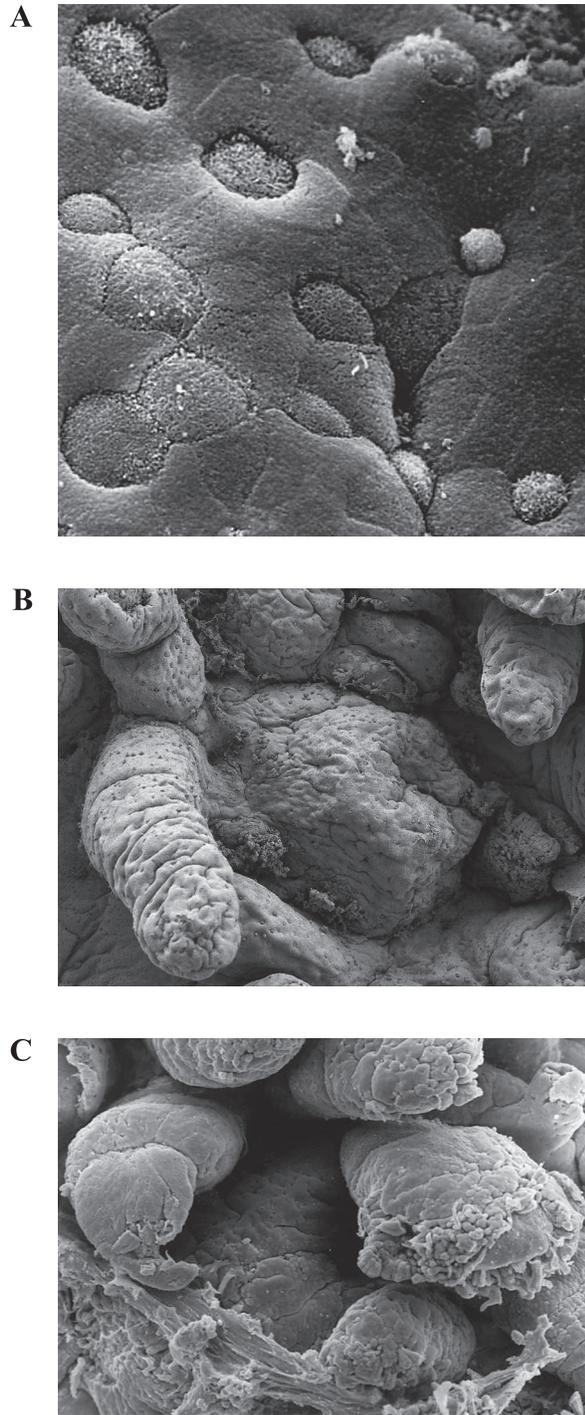
Fig. 5. Transition electron microscopy using a biopsy specimen taken from the dome of a Peyer's patch under magnified chromoendoscopy shows M cells in the follicle-associated epithelium (**A**, electron microscopic magnification, ×1800). Although the surrounding villi are atrophic and scattered before prednisolone-mesalazine therapy (**B**, electron microscopic magnification, ×100), the villi can be seen to be longer and thicker following remission (**C**, electron microscopic magnification, ×100).

exposed to luminal antigens and infectious agents, for which M cells function as a portal of entry [3, 4]. M cells are located in the FAE, but only 10% of the FAE consists of M cells [16]. Thus, it is difficult to obtain M cells using conventional endoscopic biopsy. Our experience indicates that target biopsy under magnifying endoscopy and chromo-endoscopy is beneficial for accurate biopsy sampling to obtain M cells, because this allows us to clearly recognize the FAE located on the PPs' domes. In fact, on electron microscopy, M cells were detected exclusively in the FAE. M cells are characterized by the presence of irregular cell-surface microvilli [3, 16], as clearly shown by transition electron microscopy in Fig. 5A.

In conclusion, magnifying chromoendoscopy revealed that PPs' dome irregularity and surrounding villous atrophy are common even in UC patients. Minimal mucosal lesions were seen within the PPs that had abnormal features despite the lack of an association with backwash ileitis. In addition, M cells could be detected in the FAE located on the PPs' domes using electron microscopic examination of targeted biopsy samples targeted. Long-term follow-up studies of a larger cohort of patients may help determine the patho-genesis of ileal involvement in UC.

Conflicts of interest

No conflicts of interest were declared in relation to this article.

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