Scanning Microscopy Vol. 12, No. 3, 1998 (Pages 495-502) 0891-7035/98$5.00+.25
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

SCANNING ELECTRON MICROSCOPY IN CHILDHOOD INFLAMMATORY BOWEL DISEASE

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(Received for publication November 1, 1995 and in revised form January 15, 1997)

Abstract

We have performed a study by scanning electron microscopy (SEM) on the small and large bowel mucosa of 14 children with Crohn's disease, ulcerative colitis, or indeterminate colitis. In the ileum of children with Crohn's disease, bridges between villi described in adult patients were not found suggesting that this aspect does not appear early in the development of the mucosal lesion. In large bowel mucosa, children with Crohn's disease showed an enlargement of the extrusion zones as the main lesion, with increased numbers of structurally normal mucus cells and elevated numbers of microorganisms. In ulcerative colitis , the mucosal organization was altered with a reduction in the number of crypt openings and the gland lumens were dilated. The goblet cells were reduced in number and often showed mucus containing a filamentous component. The glandular lumen contained material composed of a network of filaments. The patients with indeterminate colitis displayed ultrastructural lesions similar to those found in ulcerative colitis but the density of goblet cells was increased in large areas. In conclusion, SEM examination shows that the lesions in childhood inflammatory bowel diseases are only in part similar to those described in adults. This technique could be useful in differential diagnosis between Crohn's disease and ulcerative colitis.

Key Words: Crohn's disease, ulcerative colitis, indeterminate colitis, terminal ileitis, ultrastructure, scanning electron microscopy.

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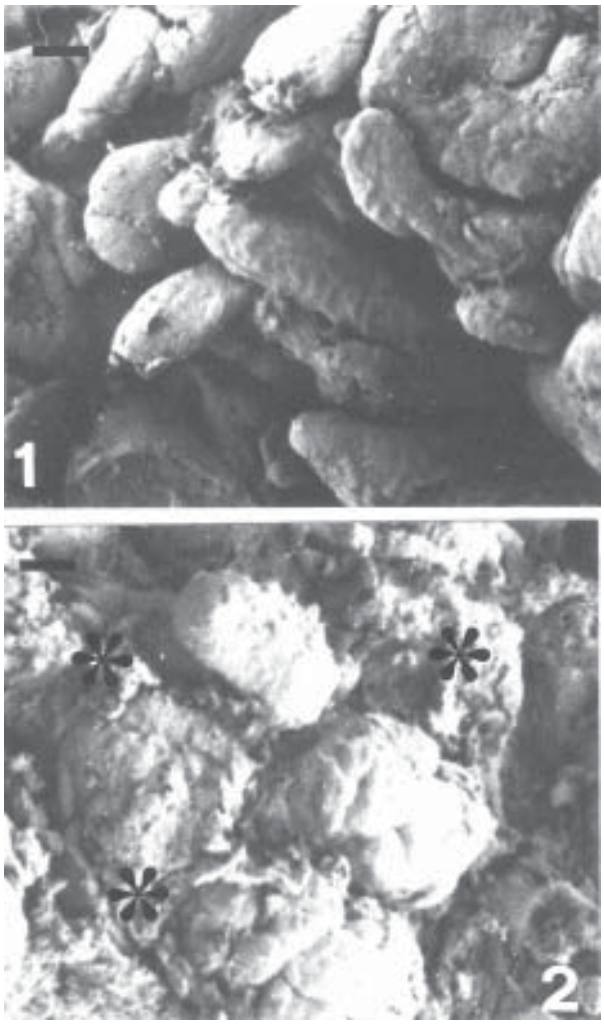
Introduction

In childhood inflammatory bowel diseases (IBD) a definitive histological diagnosis may be difficult if not impossible. Such cases are often labelled "unclassified" or "indeterminate" [10]. Morphometrical or histochemical evaluations have been proposed to improve histological studies. An alternative approach could be to extend at an ultrastructural level the study of the pathologically damaged tissue. In particular, scanning electron microscopy (SEM) has given important contributions to the understanding of mucosal damage in several gastroenterological diseases and this technique has also been applied to IBD in adult patients [1, 3, 7-9, 13]. SEM allows a three-dimensional study of the luminal surface. The analysis of the organ surface provides information on epithelial lesions and presence of mucus and microorganisms. However, to our knowledge no SEM data are available on IBD in children. This absence of studies is surprising considering the prevalence and social relevance of IBD. The present study has been performed on pediatric patients with IBD in which fragments of small or large bowel mucosa have been studied by SEM. The aim of the work was to evaluate if the ultrastructural lesions in children show some differences with respect to adults and to obtain new data on IBD in children that could be useful in differential diagnosis between Crohn's disease (CD) and ulcerative colitis (UC).

Materials and Methods

The study has been performed on the small and large bowel mucosa of 14 pediatric patients (age range 5-16 years, mean 11 years) in which the diagnosis of IBD had been made on the basis of previous clinical, radiological, endoscopic, and histological evaluations. The final diagnosis was CD in 7 cases, UC in 5 cases, and indeterminate colitis (IC) (with one suspected for UC) in 2 cases. These patients were selected because their histological lesions were characteristic of the respective diseases. The bioptical (11 cases) or surgical (3 cases) specimens were fixed in formaldehyde-picric acid and processed for standard histologic methods. The tissue blocks showing the lesions judged most representative were immersed overnight in xylene to remove the paraffin according the technique of Lager and Landas [5], washed in acetone, critical



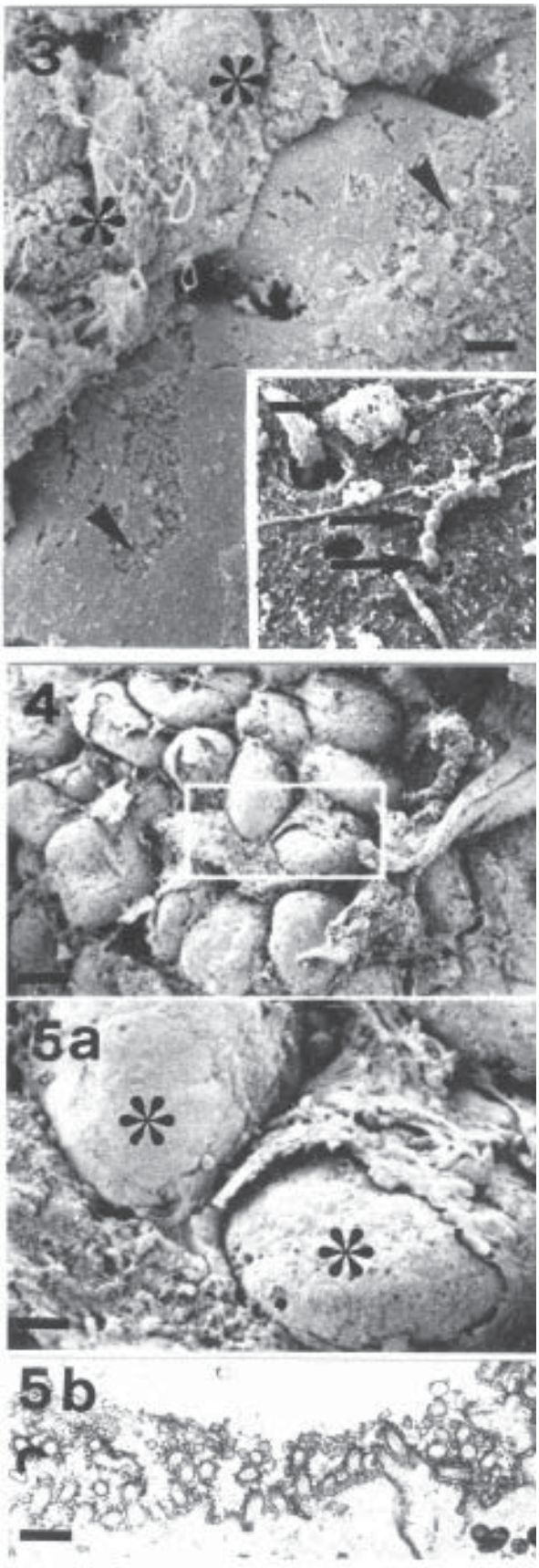


Figure 1. CD, terminal ileitis. At histology, abnor-malities consistent with partial villous atrophy were found. The villi are swollen and focally sub-atrophic. Bar = 260 μm.

Figure 2. CD, terminal ileitis. The mucosal surface is more irregular than in Figure 1, and covered by cell debris (asterisks). Bar = 400 μm.

Figure 3. CD, terminal ileitis. At higher magnification, enterocytes show loss of the glycocalyx (arrowheads). The extrusion zone at the apex of a villus is expanded (asterisks). Bar = 4 μm. The inset shows bacteria (arrows) at the mucosal surface. Bar = 2 μm.

Figure 4. CD, ileo-rectal anastomosis. The mucosal surface shows swollen villi. The area in the square is enlarged in Fig. 5. Bar = 160 μm.

Figure 5. Ileo-rectal anastomosis **(a)** At high magnification, apthtoid ulcers are visible at the apex of the villi (asterisks). Bar = 40 μm. **(b)** The enterocytes show diffuse irregularity of the microvilli. TEM. Bar = 0.4 μm.

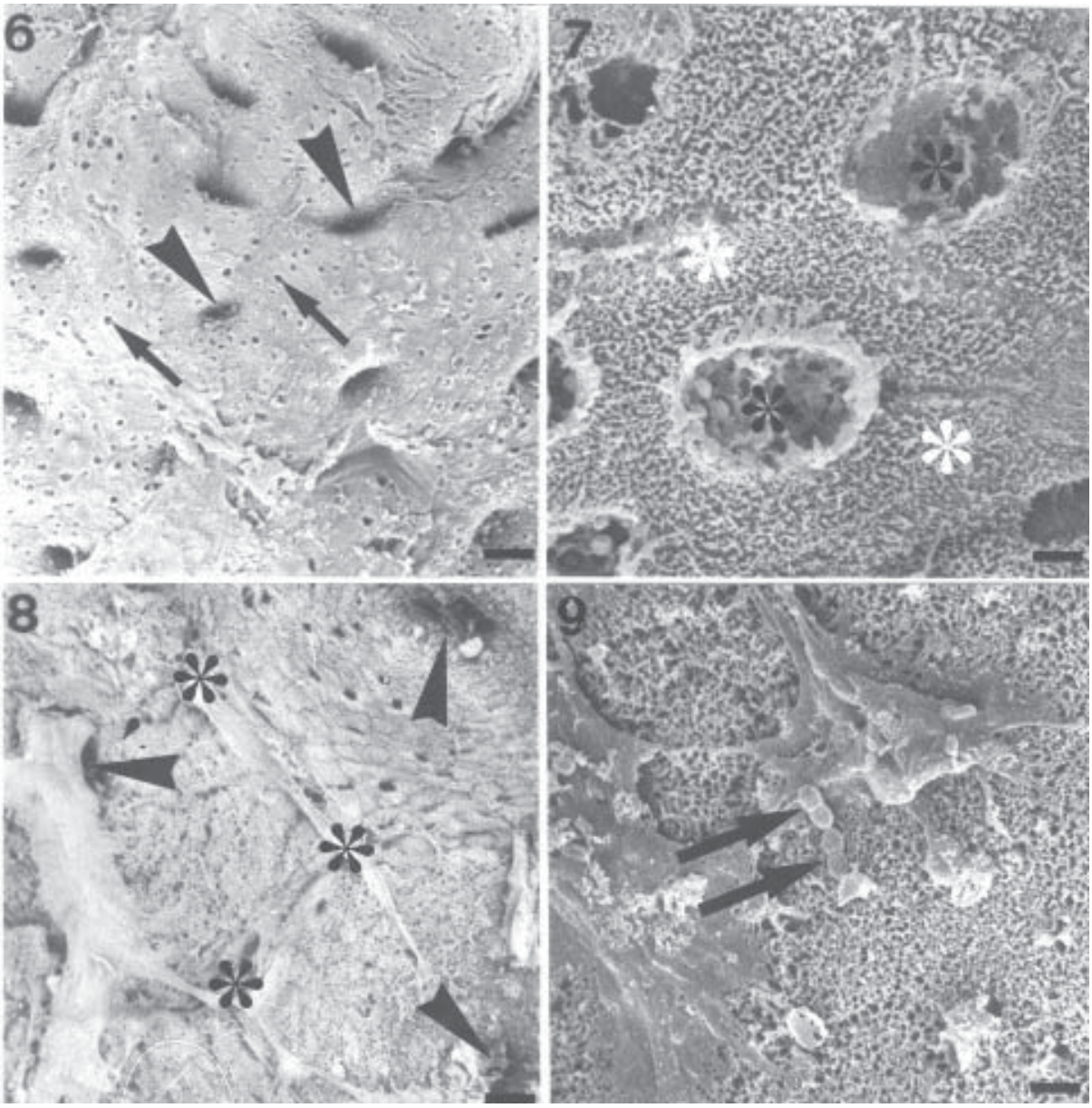


Figure 6. CD colon. At low magnification, in a histologically normal area, a regular pattern of the crypt openings is visible (arrowheads). The mucus cells (arrows) are numerous. Bar = 40 μm.
Figure 7. CD colon. Histologically normal area. At higher magnification, absorptive (white asterisks) and mucus cells (black asterisks) are visible. Bar = 3.2 μm.
Figure 8. CD colon. In areas with histological lesions typical for CD including granulomas, SEM displays a regular pattern of crypt openings (arrowheads) with enlarged extrusive zones (asterisks). Bar = 16 μm.
Figure 9. CD colon. Area with histological lesions. Bacteria (arrows) are visible. Bar = 2.7 μm.

point dried (CPD 030, Balzers, Liechtenstein), coated with gold by a sputter coater (MED 010, Balzers), and observed under a scanning electron microscope (DSM 950, Zeiss, Oberkochen, FRG) fitted with a lanthanum hexaboride cathode using an accelerating voltage of 10 kV. In all specimens standard views were obtained at low (50x, 150x), medium (500x, 1500x) and high (4000x, 10000x) magnifications. In 4 cases, non embedded (formalin or glutaraldehyde fixed) material was also examined. In these latter cases, additional specimens were postfixed in osmium tetroxide 1%, dehydrated in alcohol-acetone,

Figure 10. UC, colon. In areas without histological lesion, at low enlargement the mucosa is regular and the crypt openings are disposed in rows (arrowheads). Bar = 40 µm.
Figure 11. UC, colon. In areas with histological lesions typical for UC including crypt distortion and crypt abscesses, the mucosal organization is altered and crypt openings (arrowheads) display an irregular disposition. Bar = 57 µm.
Figure 12. UC, colon. In colectomised patients, a severe loss of the mucosal organization is visible. The luminal surface is covered with mucus and cell debris. Bar = 103 µm.

embedded in epon-araldite, sectioned by an Ultracut E ultramicrotome (Reichert, Vienna, Austria), stained with lead citrate-uranyl acetate, and observed under a transmission electron microscope (EM 10, Zeiss).

Results

Crohn's disease, terminal ileitis

At low magnification, in areas in which the histological lesions was a partial villous atrophy, the villi were swollen, shortened and irregularly shaped (Fig. 1). In areas with a more severe degree of villous atrophy at histology, SEM examination displayed a mucosa without villi and covered with cell debris (Fig. 2). At higher magnification, also in better preserved areas, the extrusion zones at the apex of the villi were expanded (Fig. 3) and numerous enterocytes showed loss of the glycocalyx and irregularity of the surface. The goblet cells were numerous and often clustered. Numerous bacteria were found in the abundant mucus. A particularly severe pattern of lesion was found in a patient with ileorectal anastomosis. The villi were preserved (Fig. 4) but showed aphthoid ulcers at their top (Fig. 5a). In the midvillar area, the enterocytes showed diffuse irregularity of the microvilli that was confirmed by transmission electron microscopy (TEM) (Fig. 5b).

Crohn's disease, colitis

In histologically normal areas, ultrastructural alterations were usually absent. At low magnification, a regular pattern of crypt openings was visible (Fig. 6) and, at higher magnification, the absorptive and mucus cells showed a normal morphology (Fig. 7).

In areas with histological lesions typical for CD including granulomas, SEM generally displayed a regular pattern of crypt openings with a diffuse oedema, an increased number of mucus cells, and degenerative aspects of the absorptive cells (Fig. 8). The extrusion zones were regularly enlarged and mucus was abundant. Bacteria were usually numerous (Fig. 9). In the glandular lumen a small amount of material was present but the deepest portion of the gland was usually devoid of material and the cells lining the gland walls had a smooth surface.

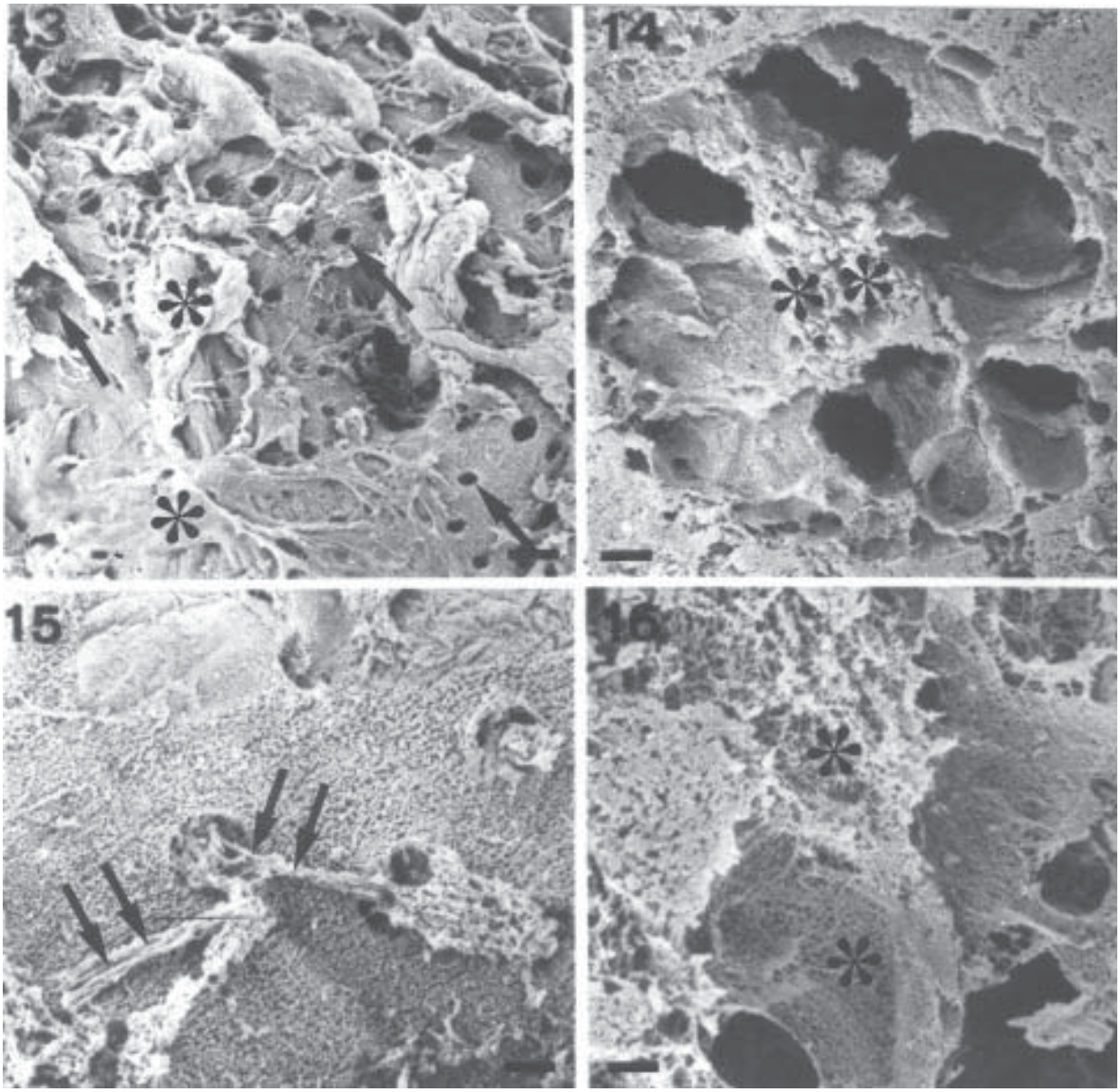


Figure 13. UC, colon. In this area, abundant mucous material (asterisks) is present at the surface and the density of goblet cells (arrows) is high. Bar = 550 µm.
Figure 14. UC, colon. Condensed material is visible in a gland lumen (asterisks). Bar = 5 µm.
Figure 15. UC, colon. The goblet cell shows mucus containing a filamentous component (arrows). Bar = 3.6 µm.
Figure 16. UC, colon. The glandular lumen contains material composed of a network of filaments (asterisks). Bar = 1.6 µm.

Ulcerative colitis

In the colon of patients with UC, a wide spectrum of ultrastructural alterations was found (Figs. 10-17). In areas without histological lesions, at low enlargement the mucosa was regular (Fig. 10) but at higher enlargement, the density of the goblet cells appeared not homogeneous (Fig. 13). In areas with evident histological lesions typical for UC including crypt distortion and crypt abscesses, the mucosal organization was altered in particular with a reduction in the number of crypt openings (Fig. 11). The glandular borders were edematous and the gland lumen was dilated (Fig. 14). The goblet cells were reduced in number and often showed a mucus containing a filamentous component (Fig. 15). Also the enlarged glandular lumen regularly contained abundant material composed of a

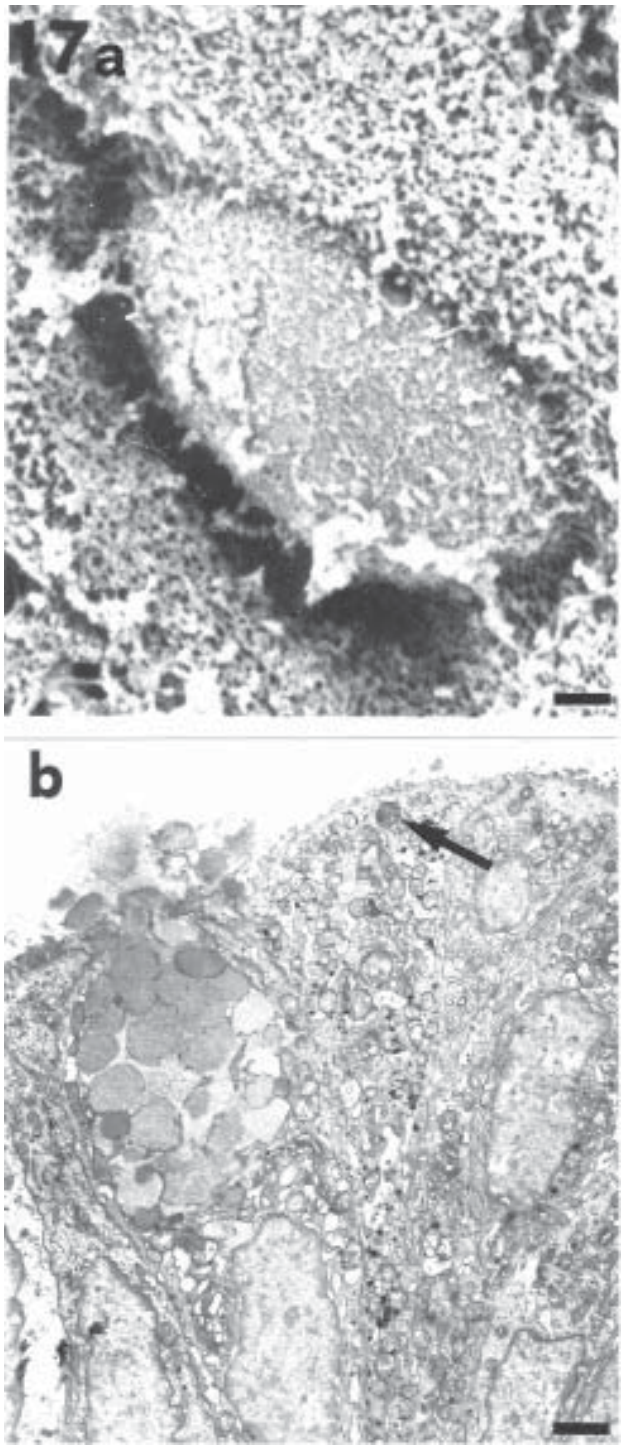


Figure 17. UC, colon. a) At SEM, a cell with sparse microvilli is visible. Bar = 1.6 µm. b) At TEM, the enterocytes display dilated cisternae of endoplasmic reticulum, short microvilli and lysosomes (arrow). Bar = 2 µm.

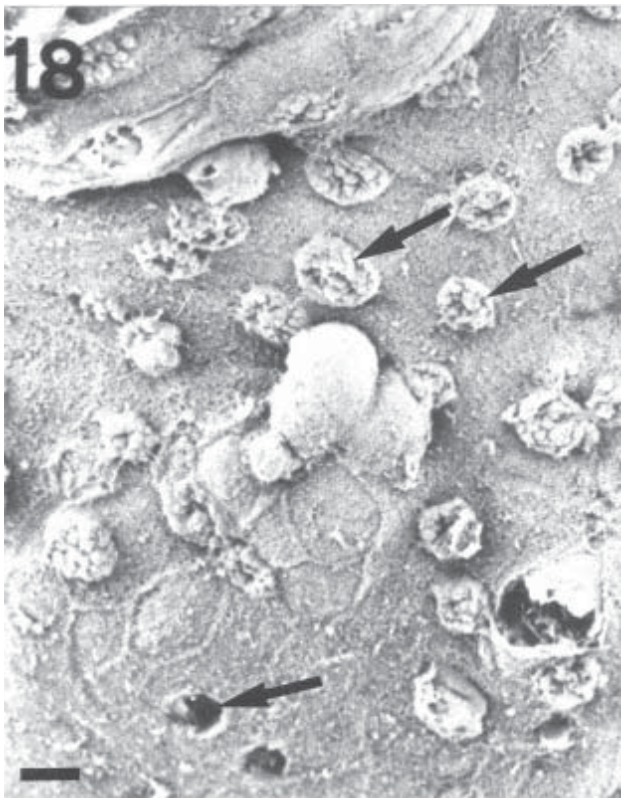


Figure 18. IC, colon. The surface shows cellular polymorphism and the density of goblet cells (arrows) is increased. Bar = 10 µm.

network of filaments (Fig. 16). This material was condensed along the axis of the gland (Fig. 14). In all the patients, numerous absorptive cells were swollen and with large apical lysosomes (Fig. 17). In some areas, the enterocytes showed an evident polymorphism and elements with sparse microvilli were found (Fig. 17). In two colectomised patients showing very active histological lesions, ultrastructural examination confirmed a loss of the mucosal organization (Fig. 12). The luminal surface was covered with mucus and cell debris. Degenerative aspects of the epithelial cells were visible.

Indeterminate colitis

Both the cases displayed widespread ultra-structural abnormalities similar to those found in patients with UC. The mucosal organization was altered in particular with a reduction in the number of crypt openings and the gland lumens were dilated. The density of goblet cells was increased in large areas (Fig. 18).

Discussion

Examination by SEM gives additional information to histology, allowing an high resolution analysis of large specimens. In the past, several studies used SEM in adult patients with UC [3, 12] or CD [4, 11]. A detailed SEM study of ileum and colon in CD was performed by Dvorak *et al*. [1]. Myllarniemi and Nickels [8] suggested that SEM can be a useful aid in the differential diagnosis of IBD. Marin *et al*. [7] used SEM, TEM and freeze-fracture to construct a model for pathophysiology of CD. Nyhlin and Stenling [9] described a high prevalence of abnormalities of the upper small-intestinal mucosa in patients with CD. Shields *et al*. [13] performed a SEM-morphometric analysis and stated that SEM may serve as an adjunct to histology in diagnosis of colonic dysplasia in patients with UC.

The present study (the first in a pediatric population with IBD) revealed in the ileum of children with CD findings similar to those described in adult patients [1] with the exception of the presence of round bacteria that are a constant finding in children with CD and were not described in adult by Dvorak *et al*. [1]. The bridges between villi described in adult patients were not found in children suggesting that this aspect is not present early in the development of the mucosal lesion.

In the mucosa of the colon, our study demonstrates that in children, CD and UC display rather different patterns of lesions. Patients with CD showed as the main lesion an enlargement of the extrusion zones, with increased number of structurally normal mucus cells and elevated numbers of microorganisms. In UC, the lesion was not restricted to extrusion zones but a more wide-spread heterogeneity of the goblet and absorptive cells was found. In children with UC there were areas revealing cells of different sizes and shapes and with a decreased number of microvilli.

In adults, Shields *et al*. [13] showed that these findings correspond to dysplastic areas at histology. The lesions of the colon found in children are only in part similar to those described in adult patients. In particular, in adults with CD goblet cells are increased in the colon surface but in UC their number is decreased [8]. In children with CD we have confirmed an increase of goblet cells. In children with UC, the reduction of goblet cells is present only in areas with severe histological lesions, while in areas marginally involved, the number of goblet cells is quite variable and can be also increased. This increase is evident in our two patients with IC as well as in the one described by Myllärniemi and Nickels [8]. Therefore, in children it seems difficult to use the number of goblet cells in the differential diagnosis between UC and CD.

The technique of Lager and Landas [5] used in the present work allowing the study of the gland wall and content at the ultrastructural level, enabled us to show the presence of mucus with a dense filamentous network in children with UC but not in children with CD or IC. This finding has not been described in previous studies on adult patients suffering from UC [3, 8, 12, 13] but in these studies the glandular contents has not been studied in detail. We have found this aspect both in superficial goblet cells and in the mucus cells of the gland. Therefore the presence of filamentous mucus seems to be interesting because it could be related to the pathogenesis of the mucosal lesion and to the formation of crypt abscesses.

In conclusion, the SEM lesions in children are only in part similar to those described in adult patients. This finding is not surprising because IBD in childhood displays distinct clinical features [2] and, moreover, by using TEM, childhood CD abnormalities have been identified that have never been described in adult patients [6]. Thus, SEM examination can give useful diagnostic information, but studies on larger series of patients are required to confirm its utility in the differential diagnosis of childhood IBD.

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Discussion with Reviewers

Reviewer I: From the Materials and Methods section it can be assumed that most of the results were obtained from samples which were recovered from paraffin blocks (10 samples were dewaxed while 4 only were taken fresh). Consequently, the risk of artifacts is very high and the authors should be aware of this.

Authors: The preservation of the ultrastructure is a difficult task and the risk of artifacts is very high in particular when dewaxed samples are used. However, in our opinion, SEM analysis of tissue blocks that had previously been examined by light microscopy is an interesting approach that allow to evaluate archives material and can give additional information with respect to standard histological methods. In particular, this approach is useful in studies on rare diseases when it is difficult to obtain fresh material. In addition, this technique seems to be the best to obtain a correlation between light microscopy and SEM and study SEM appearance of hidden structures such as the material in the gland lumen.

Reviewer II: According to Results, in UC, "The goblet cells were reduced in number..." however, in IC there are "abnormalities similar to those found in patients with UC" although "The density of goblet cells was increased". According to Discussion, also in patient with UC, areas with increased goblet cells can be found, but only in marginally involved areas. Were the areas investigated in IC patients only marginally involved?

Authors: Our technical approach allows an easy evaluation of the histologic lesion before the SEM examination, therefore, we have excluded that the areas investigated in IC patients were only marginally involved. However, our result also if obtained in a small number of cases suggest that it is difficult to use the number of goblet cells in the differential diagnosis between different clinical forms of IBD.