Microscopic colitis syndrome

B Veress, R Löfberg, L Bergman

Abstract

The colorectal biopsy specimens from 30 patients with chronic watery diarrhea but normal endoscopic and radiographic findings were studied by light microscopy, morphometry, immunohistochemistry, and two patients with electron microscopy. The histological changes in the colorectum were originally diagnosed in six patients as lymphocytic colitis and in 24 patients as collagenous colitis. The analysis of the specimens for this study could delineate three distinct groups of microscopic colitis: lymphocytic colitis (six patients), collagenous colitis without lymphocytic attack on the surface epithelium (seven patients), and a mixed form presenting with both thickening of the collagen plate and increased number of intraepithelial lymphocytes (17 patients). No transformation was seen from one type to another during follow up of six patients for four to seven years. Increased numbers of active pericryptal myofibroblasts were found with the electron microscope in one patient with mixed microscopic colitis showing also myofibroblasts entrapped within the collagen layer. Hitherto undescribed flat mucosa of the ileum was found in one patient with lymphocytic colitis and flat mucosa and thickening of the collagen plate in the ileum were seen in one patient with the mixed form of the disease. In another patient with mixed microscopic colitis, normalisation of the colorectal morphology occurred after temporary loop ileostomy, followed by the reappearance of both diarrhea, inflammation, and thickening of the collagen plate after the ileostomy was reversed. No association was found between non-steroid anti-inflammatory drug (NSAID) consumption and collagenous or mixed microscopic colitis. The primary cause of microscopic colitis is probably an immunological reaction to luminal antigen/s, perhaps of ileal origin. The engagement of the pericryptal myofibroblasts in the disease process might result in the development of the various forms of microscopic colitis. An inverse relation between intraepithelial lymphocyte count and collagen thickness may indicate that microscopic colitis is a spectral disease.

(KEYWORDS: microscopic colitis, intraepithelial lymphocytes, collagen plate, colon, collagenous colitis.)

The introduction of fibreoptic techniques in the investigation of the colorectum, combined with histopathological examination of the biopsy specimens obtained, has resulted in the recognition of subtle forms of colitis. The patients with these disorders complain of chronic, watery diarrhea, but, endoscopy is normal or shows only minimal changes, and there are no radiological abnormalities. Morphologically mild or moderate inflammation is seen in the lamina propria, combined with either thickening of the subepithelial collagenous colitis, or lymphocytic attack on the surface epithelium (microscopic colitis, lymphocytic colitis). These comparatively rare colitides are of unknown aetiology and the relation between the two forms is not clear and much debated. The question remains open whether they are separate entities with different aetiologies or if they represent different forms or parts of a panorama of one disorder. The aim of this study was to analyse and compare the histopathology of 24 cases of collagenous colitis (CC) and six cases of lymphocytic colitis (LC) to determine their relation.

Methods

PATIENTS

Altogether 30 patients have been studied. They all presented with a history of watery diarrhea. A full investigation to exclude other causes of diarrhea was carried out. The endoscopic and radiographic findings were normal in all patients. Culture of stool for pathogenic micro-organisms were negative in all patients. Of the 30 cases, 24 were diagnosed at Huddinge University Hospital and six at the Central Hospital in Karlstad. Twenty four of the patients were originally classified as CC and six as LC.

Table I gives the clinical details for the patients. In all but three patients (numbers 8, 12, 24) total colonoscopy was carried out, with multiple biopsy specimens taken from 5–10 locations, spread over the entire colorectum, with at least two specimens at each site. In patient number 24 only rigid sigmoidoscopy could be performed, with specimens taken only from the rectum. In six patients (numbers 1, 16, 17, 18, 20, 29) repeated biopsies were performed during the follow up period of 4–7 years after the diagnosis. The ileum was

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Clinical details for 30 patients with microscopic colitis</th>
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<tbody>
<tr>
<td></td>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>Group A</td>
<td>0/6</td>
</tr>
<tr>
<td>Group B</td>
<td>0/16</td>
</tr>
<tr>
<td>Group C</td>
<td>0/5</td>
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</table>
biopsied in nine patients (numbers 1, 3, 6, 7, 9, 11, 14, 15, 21), the duodenum/jejunum in a further four patients (numbers 17, 18, 21, 23), and the stomach in two patients (numbers 17, 18).

**BIOPSY PROCESSING**

The biopsy specimens were fixed overnight in 4% formaldehyde pH 7.4 and embedded in paraffin wax. At least four sections of 5 mm in thickness cut at different levels from each block were stained with haematoxylin and eosin. Further sections were stained with Van Gieson, Ladewig’s trichrome, sirius red, Giemsa, and Gram stains. The ileal biopsy specimens from two patients with flat mucosa (numbers 1, 9) were also stained with high iron-diamine and KOH-PABS methods for sulphated and sialomucins. The thickness of the collagen plate was measured at points in well oriented parts of the specimens between two crypts arranged parallel to each other.

**Evaluation of histological characteristics**

The number of intraepithelial lymphocytes, eosinophils, and neutrophils were measured by counting 300 intercryptal epithelial cells in each section while noting the numbers of inflammatory cells. At least four sections were examined in each specimen and the mean numbers (SD) of the different inflammatory cell types per 100 epithelial cell nuclei were given for the right, transverse, and left colon, and the rectum. Similar counting was performed on the sections of the ileal biopsy specimens. As a control the numbers of intraepithelial inflammatory cells in normal parts of the colon and ileum from 10 patients who had bowel resection for malignant tumours were similarly measured.

The thickness of the collagen plate beneath the intercryptal surface epithelium was directly measured by an ocular-graticule (WHK, x10/20L, Olympus Optical Co, Tokyo, Japan) in each specimen, in at least five different locations, in well oriented trichrome stained sections. The upper limit of normal for the thickness of the collagen plate was 8 μm according to our own measurements on the control material and published data.

The grade of inflammation was determined as mild, moderate, or severe. The predominant cell type was also noted.

**Results**

Table III shows the histological characteristics in each patient. Tables IV and V summarise the mean numbers of intraepithelial lymphocytes (IEL) and the mean thicknesses of the collagen plate in the right, transverse, and left colon and the rectum, respectively.

Based upon the status of the collagen plate and the number of IEL, three groups could be differentiated: group A, with heavy lymphocytic infiltrate and normal collagen plate (Fig 1A); group B, with the presence of both increased number of IEL and thickened collagen plate (Fig 1B); and group C, showing thickened collagen plate without increased number of IEL (Fig 1C). There were no significant differences found between the groups regarding the age of the patients at the onset of the disease and the number of daily diarrhoea episodes; whereas all group A patients were women, two of five group C patients were men. Only five of 30 patients had
TABLE IV  Mean numbers (SD) of intraepithelial lymphocytes in the colon in different forms of microscopic colitis

<table>
<thead>
<tr>
<th>Group of colitis</th>
<th>Colon</th>
<th>Right</th>
<th>Transverse</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>59 (35)</td>
<td>55 (23)</td>
<td>52 (12)</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>31 (15)</td>
<td>30 (16)</td>
<td>31 (11)</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>8 (2)</td>
<td>8 (2)</td>
<td>7 (2)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5 (2)</td>
<td>4 (2)</td>
<td>4 (2)</td>
<td></td>
</tr>
</tbody>
</table>

The differences between various regions of the colon within each group are not significant, p values are varied between p<0.2 and p<0.4.

A history of previous or current NSAID intake (including ASA) for other than occasional use or for longterm treatment (>6 months). Three of those five patients (numbers 14, 19, 27) only had a low dose of ASA for cardiovascular reasons and two patients (numbers 5, 7) had rheumatoid arthritis.

The surface epithelium showed patchy changes in each case, but they were more severe in groups B and C. Flatting of the epithelial cells, vacuolisation of the cytoplasm, irregular widening of the intercellular space, and partial or complete detachment of the epithelium were seen. The crypt epithelium showed no changes in any of the patients. Mild cryptitis was seen on one occasion each in a very few crypts in patients numbers 9 and 11. In patient number 1 there was one crypt abscess and one inflamed crypt in two rectal biopsy specimens at the beginning of the observation period but not later. Cryptitis, crypt abscesses, and epithelioid granulomas were not seen in the other patients. The goblet cells were normal in each case.

Electron microscopy in patient 18 showed the presence of several bacteria in the widened intercellular spaces between the epithelial cells but never in the stroma beneath the epithelial basement membrane (Fig 2). No bacterial invasion of the epithelium or lamina propria was seen in Giemsa or Gram stained sections from the other patients in the light microscopy study.

A few eosinophils and sometimes neutrophils could be found among the intercryptal surface epithelial cells, but only the numbers of IEL showed characteristic changes. Heavy or moderate IEL infiltrate was present in group A. No significant change could be seen in patient number 1 during the six year follow up. The IEL increase was moderate in group B, with the exception of patient number 7, who had severe IEL infiltration. The mean (SD) number of IEL was 50 (19) per 100 epithelial cells in group A and 34 (12) per 100 epithelial cells in group B (Table III) in contrast with the normal value of 4 (2) per 100 epithelial cells. Immunohistochemistry showed that the IELs were of T cell origin (UCHL1+, MT1+, L26+, KP1−, Mac−). There was no significant difference between the different locations of the colon regarding the number of IEL (Table IV).

No thickening of the collagen plate was seen in group A, whereas considerable thickening was present in groups B and C. The thickening was always patchy, comparatively rarely involving more than 75% of the actual specimen. The collagen plate was least thickened in the rectum, and in two of 16 group B patients it was of normal thickness in this location (Table V). In patient number 24, with maximal thickness of 60 μm, the diarrhoea started only about six months before diagnosis. Thin collagen fibres extending from the collagen plate into the lamina propria were an early sign of an abnormal plate. Of the special stains used in this study sirius red was the best staining

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**TABLE V  Greatest thickness of collagen plate in different regions of the colon and rectum within group B and group C (mean (SD))**

<table>
<thead>
<tr>
<th>Colon</th>
<th>Right</th>
<th>Transverse</th>
<th>Left</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B (n=17)</td>
<td>25 (15)</td>
<td>29 (13)</td>
<td>30 (11)</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Group C (n=7)</td>
<td>33 (14)</td>
<td>30 (15)</td>
<td>26 (10)</td>
<td>22 (16)</td>
</tr>
</tbody>
</table>

p Values within group B: right colon vs rectum = p<0.05; colon transversum vs rectum = p<0.005; left colon vs rectum = p<0.005. Within group C the differences between the various regions are not significant, p values are varied between p<0.2 and p<0.6.
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During follow up of five cases. It is noteworthy that the mean thickness in those group B patients who had more than 40 IEL/100 epithelial cells was only 24 (10) μm, in contrast with the rest of group B patients, with a mean value of 38 (9) μm.

The history of patient 16 is of particular interest. Two years after the diagnosis of collagenous colitis (collagen table 25 μm thick; Fig 3A) a resection of the sigmoid colon was performed, with a temporary loop ileostomy. Six months later multiple biopsy specimens from the whole colon showed no thickening of the collagen plate. Subepithelially there were large histiocytes and moderate chronic inflammation in the stroma (Fig 3B). The ileostomy was reversed, but immediately after the operation severe diarrhoea developed, and three months later removal of the colon and permanent ileostomy was necessary. Histological evaluation showed almost diffuse thickening of the collagen plate in the whole remaining colon, varying between 20 and 40 μm. The rectum was left behind and in biopsy specimens five and six years later normal histological pictures were seen, without inflammation or collagen thickening.

In the lamina propria there was a mixed inflammatory infiltrate, with plasma cells predominant in all cases (Table III). Lymphocytes of T cell origin were also present in fairly large numbers, but eosinophils were seldom seen in groups A and C. Neutrophil leucocytes were very rarely seen and then only in small numbers. The inflammation was mildest in group C patients. Electron microscopy in case number 18 showed two to three layers of myofibroblasts of the pericytoplasmic sheath in the upper region of the crypts containing dilated endoplasmic reticulum filled with moderately electron dense finely granulated material (Fig 4). The 'stellate' cells entrapped within the collagen plate showed also the characteristics of actively secreting myofibroblasts. In patient number 1 and in the healthy volunteer the pericytoplasmic sheath was composed of only one layer of myofibroblasts and no layer of tightly packed collagen fibrils was seen under the surface epithelium.

The ileum showed severe flat mucosa in patient number 1 of group A. In patient number 9 of group B there was both flat mucosa and thickening of the ileal subepithelial collagen plate (15 μm; Fig 5). In these two patients and in patient 3 the numbers of IEL were also increased in the ileum (Table III). When biopsied in six other cases, the ileum showed normal morphology; nor could any pathological changes be seen in the specimens from the stomach and duodenum/jejunum.

Discussion

The recently described uncommon forms of colitis characterised by watery diarrhoea but only microscopic changes are intriguing disorders. The terminology of this group of diseases is not completely clear. Whereas the term 'collagenous colitis' is well established and accepted, the other form has been called...
The confusion in diagnosis and terminology can be explained by the unclear histological criteria for LC and CC. It is noteworthy that in the original description of CC there is no mention of increased numbers of IEL, and the microphotographs show only the thickened collagen plate and stromal inflammation. There has been no mention of increased numbers of IEL in other publications. Lazenby et al in their recent paper on the diagnosis of CC stated that 'the majority of cases have a distinctive increase in intraepithelial lymphocytes'. Thus, it can be assumed that in some of their CC cases there was no increase in IEL. Nevertheless, no data are available on the proportions between these subgroups of CC patients in their publications.

The analysis of our material has confirmed the existence of three distinct histomorphological patterns, corresponding to LC (group A; six patients), to 'classic' CC without increased IEL (group C; seven patients), and to a 'mixed morphology' (group B; 17 patients). The features common to all three groups are epithelial cellular damage of varying severity, almost total absence of acute inflammation, a mixed chronic inflammation in the lamina propria, lack of cryptal changes, and normal goblet cell population. The main histological differences between LC and 'classic' CC are the normal collagen plate in the first and the normal number of IEL in the second. The thickness of the collagen plate has never exceeded 8 μm in LC in our material or in published reports.

In contrast with the data in published works, all patients with LC in our material are women. In our mixed group there is female predominance (M:F=1:8), whereas in Australia four of five patients with 'mixed pattern' were men. The female predominance among patients with 'classic' CC is in line with the previously reported data of Giardello et al.

We cannot draw any firm conclusion regarding the relation between LC (group A), mixed microscopic colitis (group B), and CC (group C) based upon our findings. On the one hand, the fairly constant histomorphology in our six patients who were repeatedly biopsied during four to seven years follow up might suggest that there is no transformation from mixed microscopic colitis to either LC or CC. The development of a 60 μm thick layer of collagen
The finding, on the other hand, that the mean thickness of the collagen plate was less (24 μm) in those group B patients who had more than 40 IEL/100 epithelial cells in contrast with the rest of group B patients (mean value 38 μm) could suggest a spectrum of the microscopic colitis. Repeated biopsies and HLA analysis of a sufficiently large number of patients would be required to answer this question.

The pathogenesis of the various types of microscopic colitis is unknown. An autoimmune reaction has been proposed, although infection has also been raised as a possibility. A possible association between CC and NSAID consumption as well as the role of abnormal pericryptal myofibroblasts in the production of the thickened collagen plate in CC have also been suggested.

Because of the histology of LC and mixed microscopic colitis, we believe that an immunological mechanism is the primary cause of these diseases. The IEL in the two types of microscopic colitis are of T cell origin according to both our investigations and those of Armes et al., and they are mainly CD8 suppressor cells. These lymphocytes recognise class I associated antigens of endogenous origin. It can be speculated that T lymphocytes are stimulated by a luminal antigen that cross reacts with an endogenous antigen expressed by epithelial cells. The other possibility is a direct autoimmune reaction against enterocytes. In this context, our finding in some cases of the occurrence of flat mucosa in the ileum accompanied by increased numbers of IEL might be of importance. These previously undescribed changes suggest a more extensive involvement of the bowel than formerly thought, and might be the result of the production of some antigenic substance/s in the ileum resulting in the damage of the ileal and colonic mucosa. The findings in patient number 16 (the normalisation of the colonic morphology after temporary loop ileostomy, the reappearance of diarrhoea, inflammation and thickening of the collagen plate after the ileostomy was reversed, and the normal rectal morphology five years after colectomy and permanent ileostomy) support this hypothesis.

According to our previous investigations, bile acid malabsorption has no role in the development of mixed microscopic colitis or collagenous colitis, although, in one of our LC patients with flat mucosa bile acid malabsorption was present. The concept of NSAID as a potential factor in the aetiology or pathogenesis of CC could not be supported in our material.

This study does not support the infectious theory. The presence of bacteria in one of the biopsy specimens of one patient was probably a secondary phenomenon following severe epithelial damage; the bacteria had not invaded the lamina propria and they had not been phagocytosed. Also, an extensive search for bacteria in the specimens of the other patients was negative. The cultures for pathogenic micro-organisms were also negative in all patients. Although a failure to find bacteria does not exclude the role of infection we believe that bacterial infection does not play a primary part in initiating the various forms of microscopic colitis.

Regarding the role of abnormal pericryptal myofibroblasts, our limited findings support the theory of their altered, active secretory function and increased number. Among the mono- kines/lymphokines there are also fibrogenetic substances. Therefore the production of such substance(s) after a primary immunologic event, and their effect on the myofibroblasts, enhancing their collagen synthesising capacity, is a possibility. The different morphologies in the three types of microscopic colitis could be the result of the absence/presence of the production of fibrogenetic monolymphokines. To test whether this hypothesis is true or false requires further studies.

The distribution of collagen thickening has important practical implications. Our study, by showing significant differences between the thickening of the collagen plate in the rectum and the rest of the colon, and by showing normal or slightly thickened collagen layers in the rectum, confirms the findings of Giardello et al., and that of Ansa and Tanaka et al. and contradicts the findings of Lazenby et al., Armes et al. The change in the collagen plate was, however, always present in the sigmoid colon, and therefore we suggest the use of at least a flexible sigmoidoscope, with biopsy specimens from rectum and with biopsy specimens from colon.

In conclusion, this study could delineate three distinct types of microscopic colitis: lymphocytic colitis, collagenous colitis without lymphocytic attack on the surface epithelium, and a mixed form presenting with both lymphocytic attack and thickening of the collagen plate. No transformation has been seen in six patients during follow up suggesting that these forms are quite stable morphologically. The primary cause of these types of colitis is probably immunological, with or without engagement of the pericryptal myofibroblasts, which might be responsible for the formation of the thickened collagen plate.

The association found between NSAID consumption and collagenous and mixed microscopic colitis. The inverse relation between intraepithelial lymphocyte count and collagen thickness may show that microscopic colitis is a spectral disease with lymphocytic colitis and collagenous colitis at the two extremes of the panorama. Further studies, some of which are already under way at our institution, are needed to consider these questions with the aim to see whether there is clinical relevance of this histological classification or not.

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