Immunohistochemical characterization of lymphocytes in microscopic colitis

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Collagenous colitis; Lymphocytic colitis; Microscopic colitis; Lymphocytes; Immunohistochemistry

Abstract

Background and Aims: Microscopic colitis (MC), encompassing the subgroups collagenous colitis (CC) and lymphocytic colitis (LC), is characterized by macroscopically normal or near-normal colonic mucosa, and an increased number of intraepithelial lymphocytes (IELs) and mononuclear cell infiltration in the underlying lamina propria (LP), in addition to an increased collagen layer in CC. This study aimed to characterize the inflammatory cells involved in mucosal inflammation, using immunohistochemistry.

Methods: Paraffin-embedded biopsies from 23 untreated patients with MC (CC=13, LC=10) and 17 controls were stained with antibodies against CD3, CD4, CD8, CD20, CD30, Foxp3, CD45RO and Ki67. Computerized image analysis was used to calculate areas of stained lymphocytes in the surface and crypt epithelia as well as in the LP.

Results: In CC and LC, an increase of predominantly CD8⁺ lymphocytes was seen in both the epithelium and the lamina propria, whereas a decreased amount of CD4⁺ lymphocytes was found in the lamina propria. CD45RO⁺ and Foxp3⁺ cells were more abundant in all areas in both patient groups compared to controls, as were CD20⁻ areas, although more scarce. Ki67⁺ areas were only more abundant in the epithelium, whereas CD30⁺ areas were more abundant in the lamina propria of both patient groups compared to controls.

Conclusions: This study confirms an increased amount of CD8⁺ lymphocytes in the epithelium. Lymphocytic proliferation and activation markers were more abundant, whereas a decreased amount of CD4⁺ lymphocytes was seen in the LP. Further studies are needed to reveal the underlying mechanism(s).

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1. Introduction

Microscopic colitis (MC) is a group of disorders previously regarded as rare, but which has emerged as a common cause of chronic diarrhea, especially in middle-aged or older women. The two most common subgroups are collagenous colitis (CC) and lymphocytic colitis (LC). CC was first described in 1976 by Lindström, and LC in 1989 by Lazenby et al. The clinical symptoms of CC and LC are similar, and the diseases cannot be differentiated on clinical grounds. Both disorders cause chronic or recurrent, non-bloody, watery diarrhea, often associated with nocturnal diarrhea, abdominal pain and weight loss. The diseases present with a macroscopically normal or near-normal colonic mucosa.

The diagnosis is based on microscopic examination of biopsies preferably from the proximal colon, showing characteristic features. The histopathological hallmarks of CC are a thickening of the subepithelial collagen layer, a chronic inflammation in the lamina propria, surface epithelial damage and sometimes increased amounts of infiltrated intraepithelial lymphocytes. In LC, a characteristic increase of lymphocytes is seen in the epithelium, the surface epithelium is damaged and chronic inflammation is present in the lamina propria.

Although the etiology of MC is unknown, both CC and LC are currently considered to represent specific mucosal reactions to various luminal agents, such as infectious agents, drugs or...
dietary factors in predisposed individuals. In the large majority of patients, however, the underlying cause is unknown.\textsuperscript{2,11} Earlier studies of both LC and CC have found that the increased number of intraepithelial lymphocytes consists predominantly of CD8\textsuperscript{+} T lymphocytes expressing the \alpha/\beta heterodimer, whereas the lymphocytes in the lamina propria were dominated by CD4\textsuperscript{+} T lymphocytes.\textsuperscript{12–14} The data are, however, based on a fairly small number of patients; the largest and most recent study from 1996 by Mosnier et al. included only 8 patients with CC and 4 with LC.\textsuperscript{14} A study in MC involving 6 patients of patients, however, the underlying cause is unknown.\textsuperscript{2,11} Previous collected, paraffin-embedded colonic biopsies were obtained from patients with LC, CC and controls from the archives at the Department of Pathology, Örebro University Hospital. The study samples included 13 CC patients (female = 9, male = 4), 10 LC patients (female = 7, male = 3) and 17 controls (female = 11, male = 6).

2. Material and methods

2.1. Study population

Previously collected, paraffin-embedded colonic biopsies were obtained from patients with LC, CC and controls from the archives at the Department of Pathology, Örebro University Hospital. The study samples included 13 CC patients (female = 9, male = 4), 10 LC patients (female = 7, male = 3) and 17 controls (female = 11, male = 6).

| Table 3 | Results of the image analysis of the immunohistochemical staining in different areas of colonic mucosa. The table presents results of immunohistochemical staining in different areas of colonic mucosa, in patients with collagenous colitis (CC) and lymphocytic colitis (LC), and controls (Ctrl). Data are expressed as the median (IQR) percent of positively stained areas. Mann-Whitney U-test was used to calculate statistical differences between patient groups compared to controls. |
|---|---|---|---|---|
| **Intraepithelial lymphocytes** | **CD3\textsuperscript{*}** | **CD4\textsuperscript{*}** | **CD8\textsuperscript{*}** | **CD20\textsuperscript{*}** |
| CC | 6.74 (4.43–14.20) \textsuperscript{**} | 0.00 (0.00–0.02) \textsuperscript{**} | 5.08 (2.44–10.91) \textsuperscript{**} | 0.04 (0.00–0.12) \textsuperscript{**} |
| LC | 22.66 (16.87–35.15) \textsuperscript{**} | 0.05 (0.00–0.33) \textsuperscript{**} | 17.16 (11.50–20.80) \textsuperscript{**} | 0.02 (0.00–0.06) \textsuperscript{**} |
| Ctrl | 3.94 (3.35–5.75) | 0.11 (0.04–0.38) | 1.8 (1.31–3.19) | 0.00 (0.00–0.00) |

<table>
<thead>
<tr>
<th><strong>Intracrypt lymphocytes</strong></th>
<th><strong>CC</strong></th>
<th><strong>LC</strong></th>
<th><strong>Ctrl</strong></th>
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<tbody>
<tr>
<td>CC</td>
<td>2.26 (1.19–4.04)</td>
<td>0.03 (0.00–0.13) \textsuperscript{**}</td>
<td>1.29 (0.54–3.73)</td>
</tr>
<tr>
<td>LC</td>
<td>7.35 (4.84–14.25) \textsuperscript{**}</td>
<td>0.02 (0.01–0.10) \textsuperscript{**}</td>
<td>3.47 (2.40–7.64) \textsuperscript{**}</td>
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<td>Ctrl</td>
<td>1.83 (0.30–2.29)</td>
<td>0.23 (0.09–0.39)</td>
<td>0.79 (0.57–1.40)</td>
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<th><strong>Lamina propria lymphocytes</strong></th>
<th><strong>CC</strong></th>
<th><strong>LC</strong></th>
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<tbody>
<tr>
<td>CC</td>
<td>10.44 (7.47–15.86) \textsuperscript{*}</td>
<td>0.46 (0.08–1.96) \textsuperscript{**}</td>
<td>3.89 (2.25–5.96) \textsuperscript{**}</td>
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<tr>
<td>LC</td>
<td>24.63 (14.56–31.14) \textsuperscript{**}</td>
<td>1.36 (0.17–6.46) \textsuperscript{*}</td>
<td>6.12 (3.97–12.12) \textsuperscript{**}</td>
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<tr>
<td>Ctrl</td>
<td>8.74 (6.11–10.03)</td>
<td>5.65 (3.34–7.31) \textsuperscript{**}</td>
<td>1.95 (1.42–3.64)</td>
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<tr>
<th><strong>Upper lamina propria lymphocytes</strong></th>
<th><strong>CC</strong></th>
<th><strong>LC</strong></th>
<th><strong>Ctrl</strong></th>
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<tbody>
<tr>
<td>CC</td>
<td>9.95 (5.77–16.36) \textsuperscript{*}</td>
<td>0.12 (0.02–1.40) \textsuperscript{**}</td>
<td>4.26 (2.43–5.37) \textsuperscript{**}</td>
</tr>
<tr>
<td>LC</td>
<td>28.27 (16.83–37.24) \textsuperscript{**}</td>
<td>1.61 (0.20–8.99) \textsuperscript{P\textsubscript{0.063}}</td>
<td>8.27 (4.56–12.91) \textsuperscript{**}</td>
</tr>
<tr>
<td>Ctrl</td>
<td>8.01 (6.26–10.56)</td>
<td>6.07 (3.84–9.79) \textsuperscript{**}</td>
<td>2.06 (1.47–4.25)</td>
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<th><strong>Lower lamina propria lymphocytes</strong></th>
<th><strong>CC</strong></th>
<th><strong>LC</strong></th>
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<tbody>
<tr>
<td>CC</td>
<td>10.80 (8.05–15.13) \textsuperscript{*}</td>
<td>0.95 (0.10–2.49) \textsuperscript{**}</td>
<td>3.62 (1.93–6.17) \textsuperscript{**}</td>
</tr>
<tr>
<td>LC</td>
<td>17.00 (9.07–24.95) \textsuperscript{**}</td>
<td>0.89 (0.13–3.30) \textsuperscript{*}</td>
<td>3.81 (1.97–9.61) \textsuperscript{*}</td>
</tr>
<tr>
<td>Ctrl</td>
<td>8.04 (6.38–9.92)</td>
<td>4.19 (2.58–5.88)</td>
<td>1.86 (1.30–3.20)</td>
</tr>
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\*P < 0.001,\textsuperscript{2,11} \*P < 0.01,\textsuperscript{2,11} \*P < 0.05.
considerably, as seen in Table 1, especially in the CC group. Duration of symptoms before the diagnosis was median 10 months in CC (range 3–33) and 5 months in LC patients, but varied considerably, as seen in Table 1, especially in the CC group.

2.1.2. Controls

The 17 control cases had undergone colonoscopy for investigation of intestinal bleeding (n = 7), and/or changes in bowel habits (n = 11). Three of the controls with changes in bowel habits suffered from diarrhea-dominant symptoms. Median age at colonoscopy was 63 years (range 17–85 years).

No macroscopic changes were seen during colonoscopy, except occasional hemorrhoids and small polyps. Biopsy specimens were obtained from proximal, transverse and distal parts of the colon as routinely performed at our hospital. The pathologist also judged whether there seemed to be enough material to complete staining and image analysis. Only the paraffin-embedded blocks with biopsy specimens from the proximal colon were used, each usually containing 4 biopsy specimens.

Histopathological criteria for CC diagnosis were

1) a diffusely distributed and thickened subepithelial collagen layer of ≥10 micrometers (μm),
2) epithelial damage such as flattening and detachment,
3) inflammation in the lamina propria with mainly mononuclear cells, and
4) possible presence of increased numbers of intraepithelial lymphocytes (IEL).

Histopathological criteria for LC were

1) ≥20 IEL per 100 surface epithelial cells,
2) epithelial damage such as flattening and detachment, and
3) inflammation in the lamina propria with mainly mononuclear cells.

2.2. Histopathological examination

Routine histological sections of all biopsy specimens were re-evaluated by an experienced gastro-pathologist (SE) to verify the diagnosis of MC or, in the control cases, to ascertain that the tissues used showed no signs of inflammatory or other disease. The pathologist also judged whether there seemed to be enough material to complete staining and image analysis. Only the paraffin-embedded blocks with biopsy specimens from the proximal colon were used, each usually containing 4 biopsy specimens.

Histopathological criteria for CC diagnosis were

1) a diffusely distributed and thickened subepithelial collagen layer of ≥10 micrometers (μm),
2) epithelial damage such as flattening and detachment,
3) inflammation in the lamina propria with mainly mononuclear cells, and
4) possible presence of increased numbers of intraepithelial lymphocytes (IEL).

Histopathological criteria for LC were

1) ≥20 IEL per 100 surface epithelial cells,
2) epithelial damage such as flattening and detachment, and
3) inflammation in the lamina propria with mainly mononuclear cells.

2.3. Immunohistochemistry

Sections of formalin-fixed, paraffin-embedded tissues 4-μm thick were mounted and deparaffinized according to standard laboratory procedures. As far as possible, consecutive sections were used for the different stainings.
Before immunostaining, the sections were pretreated by placing them in a Tris-EDTA buffer (10 mM Tris, 1 mM EDTA) pH 9.0 and heated for 30 min in a microwave oven at 650 W.

Slides were stained with the following antibodies: anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-CD30, anti-Ki67, anti-CD45RO and anti-Foxp3. Manufacturers, clones and dilutions are presented in Table 2. The stainings were performed in a routine laboratory (Department of Pathology, Örebro University Hospital) using an automated immunostaining instrument (DAKO TechMate™ 500 Plus), the Dako REAL Detection System Peroxidase/DAB+ and Rabbit/Mouse staining kit (all by DAKO, Glostrup, Denmark). Hematoxylin was used as counterstain.

2.4. Morphometry

2.4.1. Imaging

Bright field images were captured from each stained slide using a microscope with a ×20 objective lens (Leica DMRXA 2) equipped with a digital camera (Leica DFC 330 FX; both Leica Microsystems, Wetzlar, Germany) connected to a computer (Dell Optiplex GX280, Round Rock, TX, USA). Image acquisition was conducted using a Windows-based image analysis tool kit, Leica QWin (Leica Microsystems), and all captured images were saved in raw TIFF format. The microscope was calibrated and a shading correction was applied to all images, thereby reducing the noise. White balance was performed on every slide before acquiring the first field of vision. Between 4 and 15 (mean 10.7, median 11) unique fields of vision were acquired from each slide. Lymphoid follicles were excluded with the margin of one field of vision on each side. If fewer than 4 fields could be achieved, the slide was excluded from analysis. The images were 1040×1392 pixels with a pixel resolution of 0.499 μm, resulting in an area of 519×695 μm for each field of vision.

2.4.2. Semi-automated image analysis

By using the macro program Leica QUIPS (Leica Microsystems), which interfaces with the Leica QWin, a sequence of instructions for repetitive image analysis was set up. Using thresholds for color intensity defined on the first field of vision on every slide, background areas and areas of stained lymphocytes were measured within the surface epithelia, crypt epithelia and the upper and lower half of lamina propria (Fig. 1). To define the basal membrane of the epithelium, the crypts and the lamina propria, and to exclude falsely marked areas, a digital A5-sized graphics tablet (Bamboo, Wacom Ltd., Tokyo, Japan) was used to manually mark out these areas. After measuring all available fields, the percentages of stained cells within the different areas were calculated. All of the analyses for each stain were performed by a single person (CG: CD3, CD4, CD8, CD20 and CD30; AK: Ki67 and CD45RO; AB: Foxp3).

2.4.3. Point counting

In order to verify the accuracy of the image-analysis routine, the number of immunostained CD4+ and CD8+ T lymphocytes in the lamina propria were analyzed in 10 unique fields of vision from each slide, using a bright field microscope, Olympus BX41 (Olympus Corporation, Tokyo, Japan), equipped with a ×40 objective lens and a 10 × 10 mm grid. Both analyses were performed by the same person (NN).

2.5. Statistical analysis

Data analyses were performed using SPSS 17.0 for Windows. Spearman correlation analysis was performed to compare the results from the semi-automated image analysis and point counting. To calculate the significance of differences between all three groups, Kruskal–Wallis one-way ANOVA was used. The Mann–Whitney U-test was used for comparisons between groups. A p value of <0.05 was considered significant.

2.6. Ethical considerations

This study was approved by the Uppsala Regional Ethical Review Board, Sweden.

3. Results

The results of the image analysis of the immunohistochemical staining are presented in Table 3. Kruskal–Wallis analyses showed significant differences between all three groups for all stains, except for CD30, which did not show significance in intraepithelial and intracrypt areas, and for Ki67 where only the intraepithelial area showed statistically significant changes. The results of point counting for CD4+ and CD8+ T lymphocytes in lamina propria are presented in Table 4.

Among the total of 320 slides stained, 36 slides were excluded due to inappropriate staining or insufficient amounts of material for the minimum numbers of fields of vision. Of the excluded slides from CC patients, four were stained with anti-CD20, three with anti-CD30, seven with anti-Ki67, four with anti-CD45RO and three with anti-Foxp3. For the LC patients two slides stained with anti-CD20, one with anti-CD30, five with anti-Ki67, two with anti-CD45RO and one with anti-Foxp3 had to be excluded, and finally for controls one slide stained with anti-CD30, one with anti-Ki67, one with anti-CD45RO and one with anti-Foxp3 were excluded.

No significant correlation between duration of symptoms and values obtained for the different stains could be observed.

Correlation analysis comparing the results from image analysis and point counting showed a correlation coefficient of 0.83 (P<0.01). A graph presenting the correlation between image analysis and point counting are seen in Fig. 2.

3.1. Increased amount of intraepithelial lymphocytes were predominantly CD8+

A significant increase of the area of CD3+ T lymphocytes was found in the epithelium in both LC and CC patients compared to controls, with the most profound rise in LC patients (Table 3). The T lymphocytes were predominantly CD8+, as illustrated in Fig. 3. The CD4+ T lymphocytes in the epithelial area in controls were not abundant and were even more scarce in the patient groups, although this did not reach statistical significance for the LC patients. Both Ki67+ and CD45RO+ areas were significantly more abundant in both patient groups compared to controls. A slight increase of the area of CD20+B lymphocytes and Foxp3+ cells was seen among the patients, whereas CD30−B and T cell areas in the epithelium were hardly seen in any of the groups.
### Table 4  
Point count, cells/field of vision. Results of point counting for CD4+ and CD8+ lymphocytes in lamina propria, analysed in 10 unique fields of vision from each slide, using a bright field microscope with a ×40 objective lens and a 10x10 mm grid. A correlation analysis comparing the results from the image analysis (Table II) and point counting showed a correlation coefficient of 0.83 (P<0.01).

<table>
<thead>
<tr>
<th>Lamina propria lymphocytes</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>1.5*</td>
<td>3.0**</td>
</tr>
<tr>
<td>LC</td>
<td>3.6*</td>
<td>5.6**</td>
</tr>
<tr>
<td>Ctrl</td>
<td>5.3</td>
<td>2.2</td>
</tr>
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</table>

* P<0.05 (vs. controls).

** P<0.01.

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### 3.2. Decreased amount of CD4+ lymphocytes in lamina propria and crypts

A significant increase in the amount of CD3+ T lymphocytes in the lamina propria for both LC and CC patients was seen compared to controls (Table 3). Part of this increase could be assigned to a concomitant increase in the amount of CD8+ T lymphocytes. In contrast, a significant decrease in the amount of CD4+ T lymphocytes was seen in both CC and LC patients (Fig. 4).

Increased areas were recorded for CD20+ B lymphocytes, CD30+ B and T lymphocytes, and CD45RO+ and Foxp3+ lymphocytes compared to controls for both patient groups, whereas no significant differences between MC patients and controls were found for Ki67+ areas.

In LC there was a significant difference between the upper and lower lamina propria with an increased amount of stained CD3+, CD4+ and CD8+ T lymphocytes in the upper compared to the lower lamina propria (Table 3). This correlates to the controls, where there was a significantly larger area in the upper lamina propria stained for CD4+ and CD8+ T lymphocytes. In contrast, in CC patients there was a significant decrease in the area stained for CD4+ T lymphocytes.

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### 4. Discussion

Knowledge about the immunopathological mechanisms behind MC is still limited. Previous studies on MC have found increased numbers of CD8+ T lymphocytes in the epithelium, 12–14,21 and the present study supports these results. However, Armes et al. state in their analysis from 1992 that CD4+ lamina propria T lymphocytes...
lymphocytes were more numerous than CD8⁺ lamina propria T lymphocytes in CC patients. This was also stated in a study by Mosnier et al. from 1996 on patients with MC. In contrast, we found decreased amounts of CD4⁺ T lymphocytes in the lamina propria of both CC and LC patients compared to controls. In accordance with this finding, a recent flow cytometry-based study by our group, conducted on a different subset of MC patients, also showed reduced proportions of CD4⁺ lamina propria T lymphocytes in MC patients compared to controls.

The reasons for the conflicting results on CD4⁺ T cell levels in lamina propria are unclear. The fact that neither Armes nor Mosnier present quantitative data for CD4⁺ cells also makes it more difficult to interpret their statements. It is also unclear whether the patients in the previous studies were on treatment or not. The patient biopsies used in our study were all from untreated patients. Since anti-inflammatory treatment, and especially corticosteroids, can alter the immune parameters investigated, this could perhaps be of importance when comparing the results. In the normal colonic wall the vast majority of T lymphocytes in the epithelium are CD8⁺, whereas most T lymphocytes in the lamina propria are CD4⁺. Our results in the control group are in accordance with this.

The LC patients and controls had more inflammatory cells in the upper compared to the lower part of the lamina propria. Since the immune system in the gut is mainly directed at defending the host against luminal antigens, this is not surprising. In CC patients the results were less obvious, and we even observed a decrease in the amount of CD4⁺ T cells in the upper lamina propria. However, this is most likely due to the collagen band taking up some of that area.

There was a fraction of CD3⁺ T lymphocytes that apparently was negative for both CD4 and CD8. One possible explanation for at least part of this difference might be that the polyclonal CD3 antibody stains the lymphocytes with a higher intensity than the monoclonal antibodies used to detect CD4 and CD8. However, the design of the computerized image analysis took this into consideration, making it unlikely that this would explain the entire variance. Part of the difference could possibly reflect the fact that a fraction of the T cell receptor αβ⁺ or γδ T cells in the epithelium normally expresses neither CD4 nor CD8, and that there is an increase of these cells in the lamina propria of the MC patients, whereas the proliferation in the epithelium is more dominated by CD8⁺ T lymphocytes, resulting in a greater proportion of stained areas compared to that found in controls. The antibodies used in this study are all extensively used at the Department of Pathology and are thus thoroughly tested.

Traditionally, observer-dependent, semi-quantitative methods such as point counting are used for immunohistochemical evaluation. This gives limited information on the relation between stained cells and the surrounding area. Computerized image analysis offers a technique for quantifying areas of interest, in this case, immunostained cells, in relation to different subsections of the colonic wall. The image analysis was conducted on digital images taken of the specimen and based on software that can be used on a standard computer. The software was programmed to see positively stained areas based on predefined limits or thresholds. By counting the number of pixels with higher color intensity than the predefined value, the computer can calculate the stained area. Due to the computer’s inability
to detect which part of the image to include, manual involvement is still needed. However, this offers the possibility to divide the tissue into separate areas of interest, as in this study.

The stained area represents the amount of antigen present in the specimen, provided the antibody is specific, and the correct dilution is used. The amount of the different surface markers probably varies on individual lymphocytes, and thereby theoretically affects the staining intensity. However, the lymphocytes were mostly seen as completely stained cells, irrespective of antibody specificity, and there was good correlation between the results in point counting and computerized image analysis.

To our knowledge there is only one study quantifying CD20+ B lymphocytes in microscopic colitis prior to the present study. Whereas Sapp et al. did not find any CD20+ IELs in either the MC patients (6 LC, 4 CC) or the controls (10), our results imply a slight increase of the CD20+ B cell population in the epithelium. Further, Sapp et al. stated that within the lamina propria, CD20+ lymphocytes were identified both within and adjacent to lymphoid follicles. In our study we excluded areas around lymphoid follicles. Despite this, we found a significant increase in the amount of CD20+ B lymphocytes in the lamina propria of both patient groups. Despite the significance, these cells were very sparse, and further studies are needed to elucidate their pathophysiological importance.

This is also the first study investigating CD30+ T lymphocytes, expressed on activated but not resting lymphocytes. The increase in the amount of CD30+ B and T lymphocytes in lamina propria corroborate our flow cytometric findings of increased frequencies of T lymphocytes expressing CD45RO, another activation marker. In the present study, when single antibody stainings were performed, we cannot say that the statistically significant and dramatically increased CD45RO+ area in the epithelium and lamina propria in both patient groups is solely due to an increase of activated T lymphocytes, since CD45RO is expressed also on monocytes, macrophages and granulocytes.

A recent study reported on significantly increased percentages of Foxp3+ cells in lamina propria in 12 of 19 LC patients, and 14 of 20 CC patients compared to controls. Our study confirms this result with a significant increase of Foxp3+ areas in lamina propria of both patient groups, with the most prominent increase seen in the LC patients. We also found a sparse but significant increase of Foxp3+ areas within the epithelium for both patient groups. The increase in regulatory Foxp3+ cells could be interpreted as an attempt to ameliorate the ongoing inflammation. Nevertheless, since Foxp3 is expressed on both regulatory CD4+ and CD8+ T lymphocytes, a paucity of regulatory CD4+ Foxp3+ cells could still be part of the pathogenesis and warrants further studies.

In conclusion, an increase of predominantly CD8+ T lymphocytes was seen in both the epithelium and lamina propria of CC and LC patients, whereas a decreased amount of CD4+ T lymphocytes was found in the lamina propria. We also found that the inflammatory cells were more active compared to those of controls, with a higher frequency of proliferating cells in both patient groups. The reason for this needs further study, but it can be speculated that regulation of T cells is affected and that this could play a role in the pathogenesis of microscopic colitis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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