Budesonide Treatment of Patients with Collagenous Colitis Restores Normal Eosinophil and T-Cell Activity in the Colon

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Background: The aim of this study was to assess the activity of eosinophils, neutrophils, and CD4+ as well as CD8+ T-cells in 11 patients with active collagenous colitis (CC) before and after 8 weeks of budesonide treatment (9 mg once daily) compared to 10 healthy individuals.

Methods: Clinical symptoms were recorded and intestinal biopsy samples were taken and analyzed by flow cytometry. Eosinophils with a high surface expression of CD44 and low CD9 expression were classified as activated. Neutrophil activity was assessed by their expression of CD66b, and CD69 was used as an activation marker for T-cells.

Results: All patients responded to the treatment. The eosinophils in active CC showed increased activity compared to controls. The activity was back to control levels after treatment. Neutrophils were not activated in CC patients before or after treatment. CD8+ T-cells from untreated CC patients had a lower activity than controls, and a tendency of lower activity was observed on CD4+ T-cells. After treatment, the activity was increased on both types of T-cells and was not different from controls.

Conclusions: In the present study we demonstrated that the inflammation in CC is characterized by activated eosinophils but there is no neutrophil activity. CD4+ and CD8+ T-cells are increased in numbers in active CC but, surprisingly, they had a lower grade of activity than in control subjects. The major finding of this study is that budesonide treatment restores the normal activation of eosinophils and T-cells, accompanied by clinical remission.

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Collagenous colitis (CC) is, together with lymphocytic colitis, a subtype of microscopic colitis and was first described by Lindström in 1976.[1] Clinically, CC is characterized by chronic watery diarrhea and occasional abdominal pain, distension, and weight loss.[2,3] Previously CC was regarded as rare but has recently emerged as a common cause of chronic diarrhea, especially in elderly women, with an incidence rate ranging between 5 and 6 of 100,000 individuals.[3,4] Patients with CC often have concomitant autoimmune diseases, most commonly thyroid disorders, celiac disease, diabetes mellitus, and rheumatoid arthritis.[1,2]

Macroscopically the colonic mucosa appears normal or almost normal, whereas microscopic examination of mucosal biopsies reveals characteristic histopathological changes; an abnormal thickening of the subepithelial collagen layer (>10 μm), as well as a lymphocytic infiltration of the epithelium and the lamina propria.[5] The etiology of CC is unknown[6] and the pathophysiology is poorly understood. Several mechanisms have been discussed such as autoimmunity,[7,8] bile acid malabsorption,[9] drug-induced injury,[10] infections,[11] and luminal agents of unknown origin.[12] Recently, an increased bacterial uptake has been proposed as an underlying cause for the frequent occurrence of relapses in CC.[13]

At present, budesonide has the best-documented efficacy in treating active CC[14] but there is a high relapse rate when medication is tapered or ceased,[15] suggesting that budesonide does not alter the natural history of the disease.

Data on the mucosal inflammation in CC are limited. Besides the characteristic lymphocytic infiltration of the epithelium and the lamina propria, infiltrates of eosinophil and neutrophil granulocytes can be observed in the mucosa.[16–18] The eosinophils are regarded as potent proinflammatory cells with the ability to release cytotoxic proteins such as eosinophil cationic protein (ECP) and major basic protein (MBP).[19] Furthermore, they participate in immunological events[20] and may act as antigen-presenting cells that stimulate T-cell proliferation and activation.[21]

Increased luminal levels of ECP in perfusion fluids from colon[22] and increased degranulation of MBP in colonic tissues from patients with CC[23] have been found. In addition, a reduction in rectal release of ECP was noted after oral prednisolone treatment of active CC,[24] suggesting that the eosinophils play an active role in the inflammation in CC.

Neutrophil recruitment and activation with release of myeloperoxidase (MPO) is a dominant feature in ulcerative colitis (UC),[25–28] but CC patients only have a slightly increased numbers of neutrophils in the mucosa.[29,30] Moreover, low levels of MPO have been detected in perfusion fluids from colon in patients with active CC,[22] implicating a low activity of the recruited neutrophils.

The mucosal lymphocytes in CC mainly consist of CD8+ T-cells in the epithelium and CD4+ T-cells in the lamina propria[31] and a Th-1 mucosal cytokine profile with interferon (IFN)-γ, TNF-α and interleukin (IL)-15 has been demonstrated.[32] Bonderup et al[33,34] reported a reduction in the grade of lymphocytic infiltrate in budesonide-treated patients, more prominent in proximal parts of the colon than in rectum, and a correlation with less symptoms and lower histological grade of inflammation was noted.

We have previously demonstrated the presence and activity of eosinophils and neutrophils by measurements of cell products in intestinal perfusion fluid and feces in patients with UC, Crohn's disease (CD) and CC.[22,35–37] These assays may provide a good reflection of eosinophil and neutrophil activity in intestinal disease. However, to look more closely at the different T-cells at the site of inflammation we have established a method to study the presence and activity of intestinal granulocytes and lymphocytes by flow cytometry using intestinal biopsies.[38] We also quantified the cells by immunohistochemical stainings of biopsy sections.

The primary aim of this study was to quantify and assess the activity of eosinophils and neutrophils in patients with active CC before and after budesonide treatment compared to healthy individuals by means of this method. The second aim was to investigate the activity of CD4+ and CD8+ T-cells in the same patients.

PATIENTS AND METHODS

Patients and Control Group

Thirteen adult patients (10 female and 3 male, mean age 59 years, range 22–79 years) with a previously diagnosed CC and ongoing clinically active disease, defined as stool frequency >4 per day, were consecutively included in the study performed at the Department of Gastroenterology, University Hospital, Uppsala, Sweden, between June 2004 and January 2008. The diagnostic criteria for CC were histological findings of a subepithelial collagen layer thicker than 10 μm, at least focally, and a lymphocytic infiltration of the epithelium and the lamina propria.[5] Patients treated with antiinflammatory drugs (aminosalicylates, corticosteroids, nonsteroidal antiinflammatory drug [NSAID], azathioprine, antibiotics) within the last 4 weeks were excluded. Additional exclusion criteria were celiac disease, gastrointestinal infection, previous colonic resection, and pregnant or breast-feeding women.

Eleven patients fulfilled the study, 1 dropped out before starting treatment and 1 was excluded due to technical problems with the flow cytometer. Controls were recruited among patients examined for anemia ($n = 4$) or were healthy volunteers ($n = 6$). The project was approved by the Ethical Committee of the Medical Faculty, Uppsala University, and all patients provided written informed consent.

At inclusion, patient demographic data and medical history were recorded (Table 1).

Before (at inclusion) and after 8 weeks of treatment with daily 9 mg budesonide (Entocort® 3 mg AstraZeneca, Sweden), clinical symptoms and stool frequency and consistency were recorded and an ileocolonoscopy with biopsy sampling was performed. Peripheral blood was also collected from all participants.

Collection and Preparation of Samples

During ileocolonoscopy, 4 adjacent biopsy samples were taken from each of 7 different locations in all patients and control subjects: the terminal ileum, cecum, right and left flexures of the colon, descending colon, sigmoid colon, and rectum. Two of the samples from each location were sent for histological analysis. The remaining 2 samples were immediately transferred into tubes filled with physiological saline solution at room temperature and were further processed within 1 hour.

Single-cell suspensions of biopsy cells were obtained using a loosely fit glass homogenizer and the cells were then washed twice with a buffer assigned for fluorescence-activated cell sorting (FACS) containing 0.05% NaN_3, 0.1% bovine serum albumin (BSA), and 0.4% trisodium citrate dihydrate in phosphate-buffered saline (PBS). Heparinized peripheral blood from the same individuals was hemolyzed with a 0.83% ammonium chloride solution and washed twice in the FACS buffer to obtain a suspension of blood leukocytes. Both types of cell suspensions were incubated with fluorochrome-conjugated monoclonal antibodies (mAbs) for 30 minutes at room temperature in the dark. After a final wash, the cells were suspended in 500 μL of the FACS buffer and analyzed.

Antibodies for Flow Cytometry

Mouse antihuman mAbs conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), or allophycocyanin (APC) were

TABLE 1. Baseline characteristics of patients and controls

	Patients	controls
Sex (F/M)	8/3	6/4
Age (years)*	59 (22–79)	38 (23–72)
Duration of symptoms (weeks)*	36 (3–104)	
Watery stools/day*	9 (5–17)	

*Mean (min-max)

used for all antigens. Isotype-matched control labeling was also performed, using fluorochrome-conjugated mouse anti-human IgMκ and IgG2bκ as controls for nonspecific staining. All antibodies used for flow cytometry were purchased from Becton Dickinson (BD) Biosciences/Pharmingen (San Diego, CA).

Flow Cytometry Assay

The flow cytometry assay was performed on a 2 laser FACS Calibur cytometer (BD Immunocytometry Systems, San Jose, CA). Fluorescence measurements were collected using a logarithmic amplifier; forward- and sidescatter was studied using a linear amplifier. Ten thousand cells were counted and analyzed in each sample. For data analyses, Cell Quest Pro software from BD was used.

Eosinophil and neutrophil granulocytes from peripheral blood or biopsy samples were identified as described in detail previously.[38] They were gated by their forward- and sidescatter properties and further identified by specific surface markers (Fig. 1). Eosinophils were identified as cells double-positive for CD9 and CDw125. Eosinophils with a high surface expression of CD44 were classified as activated. Another measure of eosinophil activation is the mean fluorescence intensity (MFI) of CD9 on the cell surface, where the expression is downregulated on activated eosinophils. CD15 was used as a marker for neutrophils and the MFI of CD66b was used as a measure of neutrophil activation.[39]

Lymphocytes were gated by their forward- and sidescatter properties and further identified by CD4 and CD8 antibodies. The MFI of CD69 was used as a measure of lymphocytic activation.

Immunohistochemistry

Immunohistochemical analyses were performed on biopsy samples from the right flexure of colon and from the rectum on all patients before and after 8 weeks of treatment and from 5 control subjects. A monoclonal antibody to eosinophil peroxidase (EPO) (Dept. of Medical Sciences, Clinical Chemistry, University of Uppsala, Sweden) was used to identify eosinophil granulocytes. Sections cut from wax-embedded blocks (prepared for routine histological analysis) were deparaffinized in xylene, rehydrated through decreasing concentrations of alcohol, and finally rinsed in distilled water. To expose antigenic sites and reduce background, the sections were heated in a citrate buffer using a pressure cooker. The slides were subsequently placed in an automated slide processing system (AutostainerPlus, Dako Cytomation, Glostrup, Denmark), where sections were blocked in hydrogen peroxide/methanol, washed, and stained in several steps. The antigen-antibody complex was visualized with 3,3′-diaminobenzidine (DAB) using a commercial Envision kit (Dako Cytomation) according to the instructions given in the manual. The samples were then counterstained with Mayer's hematoxylin (Histolab Products, Gothenburg, Sweden), dehydrated, and mounted. The sections were examined with an Olympus BH2-MDO microscope (Olympus Optical, Tokyo, Japan) and the examiner was blinded to patient data and treatment outcome.

Histology

The biopsies were fixed in 4% formaldehyde and embedded in paraffin. Three sections were cut at different levels and stained with hematoxylin-eosin and van Gieson. A

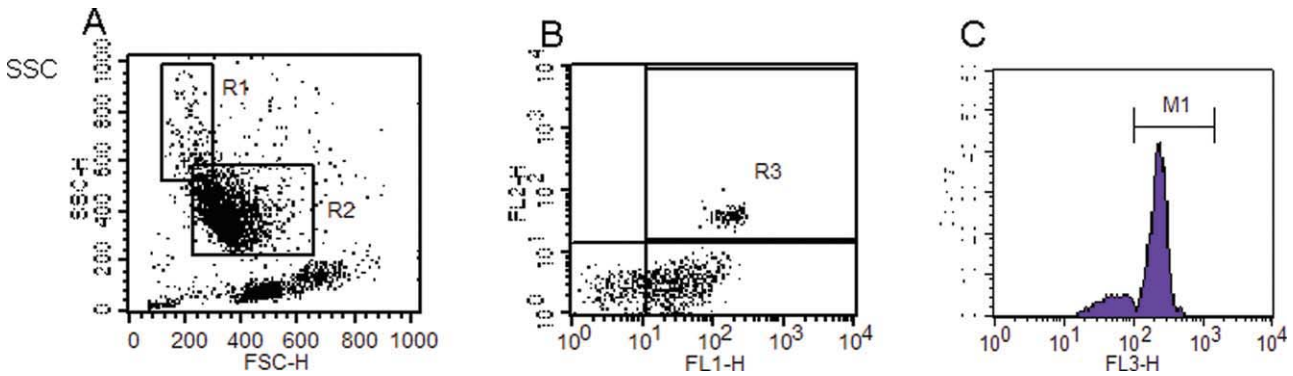

FIGURE 1. Identification gates of purified peripheral blood eosinophils (A) by forward and side scatter properties (R1) and (B) by the expression of CD9 and CDw125 (R3). (C) Eosinophils with a high expression of CD44 are gated as M1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Numbers of eosinophils per high power field, identified by immuno-histochemical staining with eosinophil peroxidase, in colonic biopsy specimens from 11 patients with CC before and after eight weeks of treatment compared to five control subjects

Location	Inclusion	Day 56	Control subjects
Right flexure of the colon	45.4 (6.8–87.1)	33.9 (17.4–60.2)*	6.8 (1.1–10.8)*
Rectum	15.4 (1.4–37.2)	8.2 (2.5–18.3)*	1.4 (0.4–3.1)*

Mean values in numbers of eosinophils identified in 10 high power fields (x340 magnification), the results are expressed as mean (min-max). Wilcoxon matched pairs test were used for the statistical evaluation comparing patients at inclusion and day 56 and Mann-Whitney U test when comparing patients at inclusion and day 56, respectively, versus control subjects.
*$p < 0.05$

4-grade scale was employed to evaluate the inflammatory response: 0 = no inflammation, 1 = light inflammation through the whole thickness of lamina propria or light inflammation only involving the luminal part of the lamina propria, 2 = heavy inflammation only involving the luminal surface of lamina propria, 3 = heavy inflammation through the whole thickness of lamina propria. Areas containing the thickest collagen band were measured with a calibrated ocular microscale and the maximum thickness and corresponding location recorded. The same pathologist reviewed the biopsies and was blinded for treatment outcome.

Statistical Evaluation

For paired analyses we used Friedman analysis of variance (ANOVA) and Wilcoxon matched pairs test. Spearman rank order correlations were used to express relationships between variables. To evaluate statistical differences between groups the Mann–Whitney *U*-test were used. A *P*-value <0.05 was considered significant. All calculations were performed on a personal computer using the statistical software Statistica (Statsoft, Tulsa, Oklahoma, USA).

RESULTS

Clinical Outcome

All patients responded to treatment and clinical remission (stool frequency ≤ 3 per day) was observed in 10 out of 11 patients after 8 weeks of treatment. One patient decreased in stool frequency from 17 to 8 but did not fulfil the clinical remission criteria.

Intestinal Eosinophil Granulocytes

Immunohistochemical staining for EPO revealed increased numbers of eosinophils in the lamina propria in patients with active CC, most obvious in the right flexure of colon, compared to control subjects. After 8 weeks of treatment the number of eosinophils decreased significantly but did not reached control levels (Table 2, Fig. 2).

We found increased eosinophil activation, assessed as decreased MFI of CD9 by flow cytometry, in biopsy samples from untreated CC patients compared to control subjects. This was statistically significant in the right flexure of the colon (Fig. 3). The CD9 expression increased after 8 weeks of treatment, indicating attenuated eosinophil activity, significant in cecum, right flexure, and descending and sigmoid colon. The MFI of CD9 after treatment was even higher compared to healthy controls in these locations.

No significant difference in the proportion of activated eosinophils, measured as high expression of CD44, was seen when comparing active or treated CC with control subjects, even if there was a tendency toward increased CD44 in active CC patients (Fig. 4).

The percentage of CD44 high eosinophils decreased after 8 weeks of treatment, significant in the left flexure and descending and sigmoid colon.

Peripheral Blood Eosinophil Granulocytes

No significant difference in markers of eosinophil activation in the peripheral blood in patients with active CC or after 8 weeks of treatment or compared to control subjects was observed.

Intestinal Neutrophil Granulocytes

No difference in neutrophil activity, assessed as MFI of CD66b, was revealed in patients with active CC compared to control subjects.

The MFI of CD66b decreased after 8 weeks of treatment, significant in the right flexure and in the descending colon ($P < 0.05$) where the expression was even lower than in control subjects ($P < 0.05$) (not shown).

Peripheral Blood Neutrophil Granulocytes

No significant difference in markers of neutrophil activation in the peripheral blood was noted in patients with active CC or after 8 weeks of treatment or compared to control subjects.

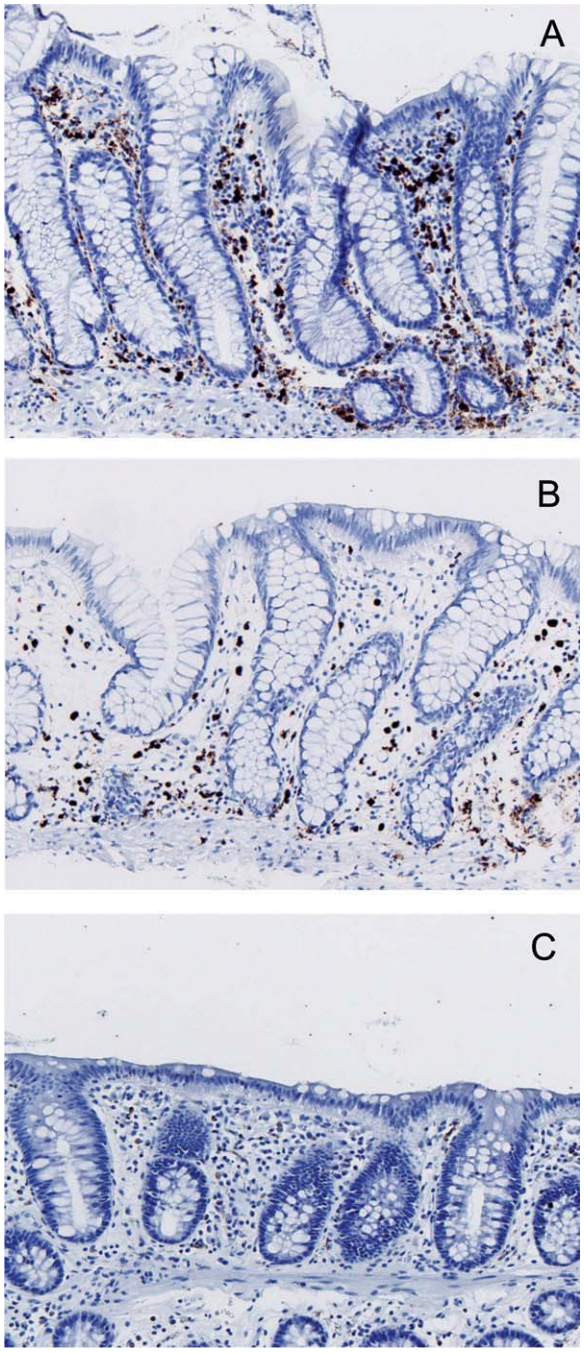

FIGURE 2. Immunohistochemical identification of eosinophils by using the eosinophil marker eosinophil peroxidase in colonic biopsy specimens from a patient with collagenous colitis before (A) and after 8 weeks of budesonide treatment (B) compared to a healthy control (C).The figure includes a representative patient and a healthy control.

Intestinal T-lymphocytes

The percentage of CD4+ T-cells (% of the total cell count) was elevated in patients with active CC before treatment compared to control subjects, significant in the right and left flexure and descending and sigmoid colon as well as in rectum ($P < 0.05$). A decline in the percentage of CD4+ T-cells was noted in CC patients after 8 weeks of treatment, significant in cecum, and descending colon and rectum ($P < 0.05$) (not shown). Surprisingly, we observed a tendency of lower CD69 expression (MFI) on CD4+ T-cells from patients with active CC compared to controls, indicating suppressed activation of CD4+ T-cells in active

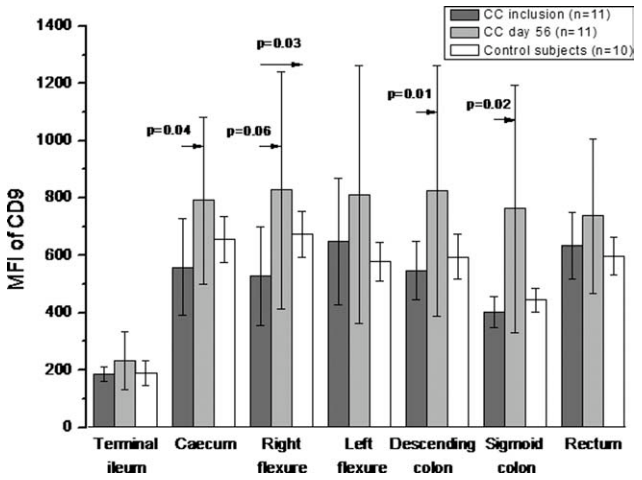

FIGURE 3. Mean fluorescence intensity (MFI) of CD9 on eosinophils from patients with active collagenous colitis (CC) before treatment (CC inclusion), after 8 weeks of budesonide treatment (CC day 56) and from control subjects. *P*-values, assessed by Wilcoxon matched pairs test (inclusion and day 56) and Mann--Whitney *U*-test (inclusion and control subjects), are indicated. The results are expressed as mean ± SEM.

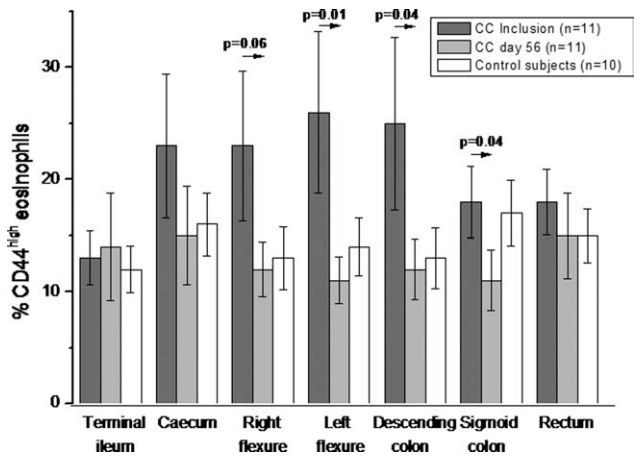

FIGURE 4. Percentage numbers of activated (CD44+high) eosinophils in patients with active collagenous colitis (CC) before treatment (CC inclusion), after 8 weeks of budesonide treatment (CC day 56) and in control subjects. *P*-values, assessed by Wilcoxon matched pairs test, are indicated. The results are expressed as mean ± SEM.

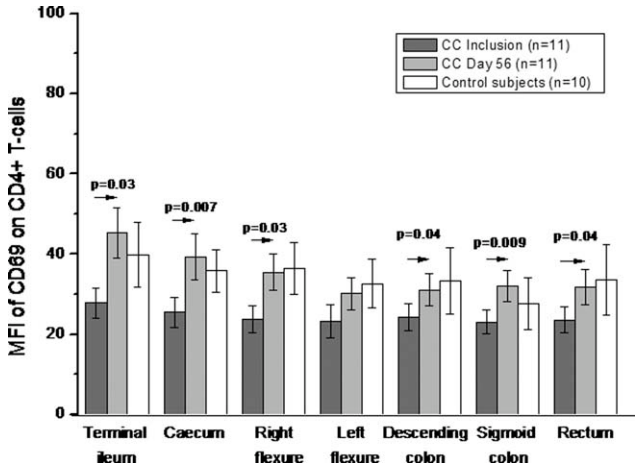

FIGURE 5. Expression of CD69 (MFI) on CD4+ T-cells from patients with active collagenous colitis (CC) before treatment (CC inclusion), after 8 weeks of budesonide treatment (CC day 56), and from control subjects. *P*-values, assessed by Wilcoxon matched pairs test, are indicated. The results are expressed as mean ± SEM.

CC. After 8 weeks of treatment the expression of CD69 increased to control levels, significant in terminal ileum, cecum, the right flexure, descending and sigmoid colon, and rectum (Fig. 5).

The percentage of CD8+ T-cells was significantly higher in the entire colon in patients with active CC as well as after 8 weeks of treatment compared to control subjects ($P < 0.001$). A decline in the percentage of CD8+ T-cells was demonstrated in CC patients after 8 weeks of treatment, significant in the right and left flexure as well as descending colon ($P < 0.05$) (not shown). The MFI of CD69 on CD8+ T-cells was lower in patients with active CC compared to control subjects, significant in right flexure and descending colon. An increased MFI of CD69 on CD8+ T-cells was observed in patients after 8 weeks of treatment, significant in ileum and sigmoid colon (Fig. 6).

Peripheral Blood T-lymphocytes

No significant difference in the percentage of CD4+ or CD8+ T-cells or in markers of T-cell activation in the peripheral blood in patients with active CC or after treatment, or compared to control subjects, was noted.

Histology

Histological findings are displayed in Table 3.

DISCUSSION

Eosinophil infiltration in the colonic mucosa is considered to be a histological feature of CC.[16–18] In the present study we confirmed these data by means of immunohistochemical staining for EPO in biopsy specimens from colon in patients with active CC. Furthermore, previous reports have shown increased eosinophil activities such as MBP release and transforming growth factor (TGF)-β production in active CC[23,40] and perfusion studies on CC patients revealed increased eosinophil degranulation during active disease.[22] In concordance with this, we found functionally activated colonic eosinophils in our patients by using flow cytometry. For the first time, we also demonstrate that the enhanced eosinophil activity decrease to control levels after 8 weeks of budesonide treatment, accompanied by clinical improvement. There are a number of reasons to consider the possibility that eosinophils are taking part in the pathogenesis of CC. Eosinophil granule proteins are toxic to intestinal epithelial cells[41] and can produce substance P and vasoactive intestinal peptide, which may alter the nervous and microcirculatory systems in the intestine.[42] Moreover, eosinophils stimulate fibroblast DNA synthesis[43,44] and express TGF-β1,[40] which stimulates fibroblast proliferation,[45] enhances collagen synthesis,[46] and inhibits the expression of the collagenase gene,[47] thereby decreasing the degradation of collagen. Animal studies have revealed that eosinophil granule proteins alter chloride ion transport[48] and increase vascular permeability.[49] Additionally, eosinophils could function as immunoregulatory cells involved in the release of both type 1 and type 2 cytokines[21] and they also have the ability to produce T-cell-attracting chemokines.[50] Therefore, eosinophils and their granule products have the capability to generate epithelial cell injury, subepithelial collagen deposition, secretory diarrhea, a type 1 cytokine profile, and chemokines that may attract T-cells to the mucosa, all characteristics in CC.

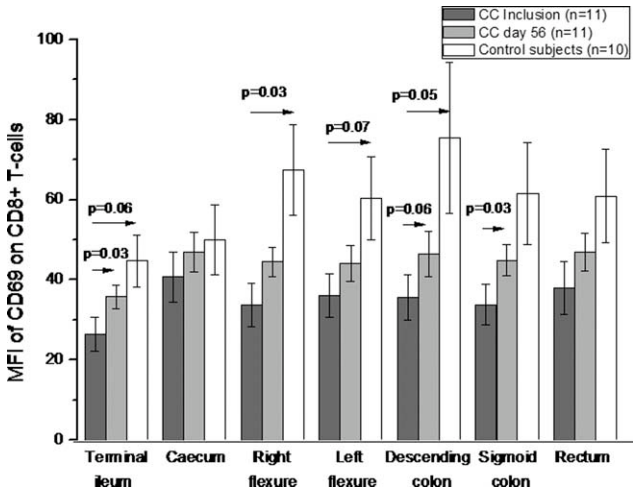

FIGURE 6. Expression of CD69 (MFI) on CD8+ T-cells from patients with active collagenous colitis (CC) before treatment (CC inclusion), after 8 weeks of budesonide treatment (CC day 56), and from control subjects. *P*-values, assessed by Wilcoxon matched pairs test (inclusion and day 56) and Mann--Whitney *U*-test (inclusion and control subjects), are indicated. The results are expressed as mean ± SEM.

TABLE 3. Histologic grade of inflammation and collagen layer thickness from colonic mucosa of patients with collagenous colitis before and after 8 weeks of budesonide treatment

Location	Inclusion n = 11	Day 56 n = 11	
Caecum	3.0 (2–3)	1,0 (0–2)	P = 0.003
Right flexure of the colon	3.0 (1–3)	1.0 (0–2)	P = 0.008
Left flexure of the colon	3.0 (1–3)	1.0 (0–2)	P = 0.008
Descending colon	3.0 (0–3)	1.0 (0–2)	P = 0.008
Sigmoid colon	2.0 (1–3)	1,0 (0–1)	P = 0.008
Rectum	2.0 (1–3)	1,0 (0–2)	P = 0.012
Collagen layer thickness (μm)*	21.8 (5–40)	17.2 (2–60)	P = ns

The grade of inflammation in the lamina propria measured semiquantitatively on a scale from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe), values expressed as median (min–max). Wilcoxon matched pairs test were used for the statistical evaluation. ns = not significant.
*Values expressed as mean (min–max)

The effects of corticosteroids on eosinophils are several, including diminished recruitment, downregulation of activation, enhanced eosinophil apoptosis, and increased tissue eosinophil clearance.[51–53] A previous prospective study on 6 CC patients reports a reduction in the numbers of eosinophils after oral prednisolone treatment,[54] in line with our results. We also revealed that eosinophil activation is down-regulated after 8 weeks of budesonide treatment. Taha et al[24] reported a decreased level of ECP in perfusion fluids from colon in prednisolone-treated CC patients, and our findings suggest that downregulation of eosinophil activity may contribute to this.

As mentioned above, CC patients only have a slightly increased number of neutrophils in the mucosa and our results imply that mucosal neutrophils in patients with active CC are not in an activated state. There was no significant difference in patients with active CC compared to control subjects. In spite of this, the activity was decreased after 8 weeks of budesonide treatment; in some parts of the colon, even lower than in controls. In a previous work we used flow cytometry to study neutrophil activity in patients with UC.[38] We found increased neutrophil activity, measured as MFI of CD66b, confirming that neutrophils participate in the inflammatory process in active UC. In contrast, during remission the neutrophils expressed a resting phenotype. In patients with active CC, neutrophil activity resembles the levels found in patients with UC in remission and display only 1/10 of levels seen in active UC (data not shown). It has been proposed that neutrophil activation may be an important aspect in severe cases of CC.[22] In contrast, the present study did not uncover any correlation in stool frequency and neutrophil activity (data not shown).

A main histopathological aspect of CC is infiltration by lymphocytes in the surface epithelium and in the lamina propria.[55,56] A previous immunohistochemical study of 8 patients with CC[31] showed an increased number of intraepithelial lymphocytes with significantly more CD8+ T-cells than CD4+ T-cells. The same study revealed an accumulation of CD4+ T-cells in the lamina propria. Our data from the flow cytometry assay reveals increased percentage of CD4+ and CD8+ T-cells in the mucosa of the colon in patients with active CC compared to controls. However, as the flow cytometry assay was performed on single-cell suspension of biopsies from colon, this method did not elucidate where the different T-cell populations were harboring in the mucosa. A decline in the percentage of CD4+ T-cells to control levels was noted in CC patients after 8 weeks of treatment. The percentage of CD8+ T-cells also displayed a significant decline after 8 weeks of treatment but was still higher than control levels in the entire colon. In line with this, the grade of inflammation, assessed by histological findings, decreased in all parts of the colon after 8 weeks of treatment but was infrequently normalized. Similar to data from Bonderup et al,[33,34] our findings confirm that budesonide treatment in CC reduce the mucosal lymphocyte infiltration. In addition to this, our data suggest that this reduction is most apparent in CD4+ T-cells, whereas the reduction in CD8+ T-cells does not reach control levels. As discussed above, eosinophil seems to play an active role in the inflammation in CC. However, there is an ongoing infiltration of T-cells in the mucosa during remission, implicating a contributing mechanism triggering the inflammation. A recent study illustrated that an increased mucosal uptake of *E. coli* 12 in CC persists after budesonide treatment, suggesting an underlying barrier dysfunction as an explanation for this.[13]

To the best of our knowledge, the present study is the first systematic investigation of lymphocyte activity in the colonic mucosa of patients with CC (before and after budesonide treatment). In a recent study of T-cell activity,

assayed by flow cytometry, in patients with different stages of IBD, tentative data indicate an increased T-cell activity in patients with active UC compared to patients with UC in remission and control subjects (Winqvist et al, in prep.).

In contrast, we observed a lower CD69 expression on both CD4+ and CD8+ T-cells in active CC compared to controls, indicating suppressed activation of CD4+ and CD8+ T-cells in active CC. The reason for this remains obscure, but this finding along with the absence of neutrophil activity imply that there is rather a smoldering than a highly active inflammation in the mucosa in active CC. During budesonide treatment, the expression of CD69 in CC patients increased to control levels, most obviously in CD4+ T-cells but also detected in CD8+ T-cells. An in vitro study of the immunosuppressive effect of budesonide on human lamina propria lymphocytes concluded an inhibitory effect on proliferation along with decreased TNF-α secretion, suggesting a downregulation of the activity.[57] The latter is contrary to our findings and one might speculate that there could be a suppressing factor influencing T-cell activity in active CC which is altered by budesonide treatment. Another hypothesis is that there might be a subpopulation of T-cells that express a downregulated activity, and that this subpopulation is reduced by budesonide treatment, eventuating in the remaining T-cells being in a normally activated state.

Our observations in the present study should be interpreted with caution, since the number of patients was small. In addition to this, all the participants responded to treatment (10 out of 11 achieved remission and 1 improved) and this denotes that our results could not obviously be interpreted in CC patients who do not respond to treatment.

In conclusion, the present study demonstrated an increased infiltration of eosinophils in the colonic mucosa, and that these cells are functionally activated in active CC. During budesonide treatment the infiltration is diminished and the activity is downregulated accompanied by clinical improvement. Mucosal neutrophils display an inactive phenotype in active CC and during budesonide treatment they become even less activated. The infiltration of mucosal CD4+ and CD8+ T-cells is increased but these cells display a suppressed activation in active CC. During budesonide treatment the infiltration of T-cells is reduced but infrequently reaches control levels and the activity is increased to levels found in control subjects, most obviously in CD4+ T-cells.

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