Journal of Crohn's and Colitis (2013) 7, 694–705

Available online at www.sciencedirect.com

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Microscopic colitis patients have increased proportions of Ki67$^+$ proliferating and CD45RO$^+$ active/memory CD8$^+$ and CD4$^+$8$^+$ mucosal T cells

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Received 26 July 2012; received in revised form 24 August 2012; accepted 24 August 2012

KEYWORDS
Collagenous colitis;
Lymphocytic colitis;
Flow cytometry;
Lamina propria lymphocytes;
Intraepithelial lymphocytes

Abstract

Background: Collagenous colitis (CC) and lymphocytic colitis (LC) are chronic inflammatory bowel disorders of unknown etiology. This study investigated phenotypic characteristics of the mucosal lymphocytes in CC and LC.
Methods: Lamina propria and intraepithelial lymphocytes (LPLs, IELs) isolated from mucosal biopsies from CC (n=7), LC (n=6), as well as LC or CC patients in histopathological remission, (LC-HR) (n=6) and CC-HR (n=4) and non-inflamed controls (n=10) were phenotypically characterized by four-color flow cytometry.
Results: The proportions of CD8$^+$ IELs were increased in CC and LC ($p<0.01$) compared to controls. Increased proportions of CD45RO$^+$CD8$^+$ IELs and LPLs were observed in LC and even more in CC patients ($p<0.01$). Both CC ($p<0.05$) and LC patients had elevated proportions of CD4$^+$8$^+$ IELs and LPLs compared to controls. The proportions of CD45RO$^+$ cells were increased in CD4$^+$8$^+$ IELs and LPLs ($p<0.05$) in CC and LC patients compared to controls. Both CC ($p<0.05$) and LC patients had higher proportions of Ki67$^+$CD8$^+$ IELs and LPLs compared to controls.
In contrast, decreased proportions of CD4$^+$ LPLs were observed in CC and LC as well as CD4$^+$ IELs in LC compared to controls. Increased proportions of Ki67$^+$CD4$^+$ IELs and LPLs ($p<0.05$) were observed in CC and LC patients.
CC-HR but not LC-HR patients demonstrated normalized proportions of both IELs and LPLs compared to CC and LC patients respectively.

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1873-9946/$ - see front matter
http://dx.doi.org/10.1016/j.crohns.2012.08.014

Conclusion: LC and CC patients have differences in mucosal lymphocyte subsets, with increased proportions of $Ki67^+$ and $CD45RO^+$ $CD8^+$ and $CD4^+8^+$ mucosal T cells.

1. Introduction

Dysregulated immune responses in the intestine can lead to chronic inflammatory conditions such as inflammatory bowel disease (IBD), and microscopic colitis (MC). Microscopic colitis comprises primarily two entities, collagenous colitis (CC) and lymphocytic colitis (LC) with an incidence rate each of 5–6 cases per 100,000 individuals per year.[1] Both conditions are characterized by chronic watery diarrhea, often associated with abdominal pain and weight loss.[1] The colonic mucosa is macroscopically normal or almost normal whereas microscopic examination of biopsies reveals distinct histopathology.[1,2] Both diseases present with increased densities of lymphocytes, but CC also has a thickened subepithelial collagen band (10 μm or more) adjacent to the basal membrane.[1,2] MC can occur at any age but most commonly affects middle-aged or elderly women; the peak incidence being around 60–65 years of age. The disease is often associated with other autoimmune disorders such as celiac disease, rheumatoid arthritis, thyroid disease, or diabetes mellitus.[1–5] The etiology of MC is supposedly multifactorial and mostly unknown, and the possible etiological and pathophysiological relationship between the two diseases is unknown. Nevertheless, the current hypothesis is that similar to IBD, MC is caused by dysregulated immune responses to a luminal agent in predisposed individuals.[1,6]As the inflammation in MC is more subtle compared to Crohn's Disease (CD) or ulcerative colitis (UC), this condition is likely an excellent "model" that can be used to study the role of basal differences in immune regulation. Strong inflammatory changes can lead to numerous and ambiguous secondary effects on the immune system. In addition, regulatory factors not present in UC and CD patients might be revealed in MC patients, holding the inflammation at bay. Such information is very relevant also for UC and CD regarding knowledge about basic pathophysiology.

The increasing but still limited immunopathophysiological data demonstrate increased numbers of $CD3^+$ T cells in lamina propria (LP) and the intraepithelial compartment of both CC and LC patients as judged by immunohistochemistry.[7] Johrens et al. reported that the majority of LP $CD4^+$ T cells from LC patients expressed the Th2 transcription factor GATA-3, whereas LP $CD8^+$ T cells expressed both the Th1 transcription factor T-bet and GATA-3 at similar levels.[8] In contrast, in the epithelium the majority of $CD8^+$ intraepithelial lymphocytes (IELs) expressed T-bet.[8] In addition, elevated mRNA levels of TNF-α, IFN-γ and IL-15 have been demonstrated in the mucosa of both LC and CC patients.[9] Besides the lymphocytic infiltration in the mucosa, increased numbers of eosinophils can be observed in CC patients,[10,11] implicating involvement of the innate immune system in MC pathology. In addition increased levels of eosinophilic cationic protein (ECP) in perfusion fluids from the colon[12] and increased degranulation of major basic proteins (MBP) were noted in colonic mucosa of CC patients.[11] Furthermore, increased levels of active NFκB in mucosal epithelial cells of CC patients have also been reported.[13] Markedly increased iNOS levels were noted in the mucosa of MC patients,[9,14,15] as well as increased levels of nitric oxide (NO), leading to increased paracellular permeability by reducing tight junction permeability.[16] Altogether the above data suggests alterations in both the innate and adaptive immune responses in MC. The nature of the adaptive local immune responses in the mucosa of MC patients is however still not fully elucidated. The primary aim of this study was therefore to phenotypically characterize the intraepithelial and lamina propria lymphocyte populations isolated from colonic biopsies from MC patients compared to non-inflamed healthy controls, with UC patients as positive controls, using four color flow cytometry.

2. Material and methods

2.1. Patients

The diagnoses of CC and LC were confirmed by characteristic clinical symptoms, ≥3 stools/day and/or abdominal pain, combined with histological analysis of biopsies according to established diagnostic criteria: LC—increased numbers of IELs (≥20/100 surface epithelial cells) in conjunction with surface epithelial cell damage and infiltration of lymphocytes in the lamina propria, but a normal collagen layer[1,17]; CC—in addition to increased numbers of lymphocytes in the epithelium and lamina propria, deposition of a ≥10 μm thick subepithelial collagen layer.[1,17] The diagnosis of ulcerative colitis was based on generally accepted criteria.[18]

Inclusion criteria included patients who were previously diagnosed with CC, LC and UC with an ongoing clinically active disease. Patients with a previous history of Crohn's disease, clinical signs of gastrointestinal infection, ischemic colitis or neoplastic disease were excluded from the study.

Colon biopsy specimens from 7 patients with CC, 6 with LC and 4 with UC were investigated. We also identified a subgroup of MC patients who were previously diagnosed with LC (n=6) or CC (n=4), but who at the time of biopsy collection showed no histological signs of inflammation despite clinical symptoms of active LC/CC. Biopsies from these patients were grouped separately, and are referred to as LC-histopathological remission (LC-HR) and CC-histopathological remission (CC-HR). We also investigated biopsies from 13 patients with diarrhea, but with histologically normal mucosa and no earlier diagnosis of MC or IBD (n=13) (hereafter referred to as diarrhea controls). Finally, 10 non-inflamed controls were recruited among patients undergoing examination for rectal bleeding or suspicion of malignancy; with a normal mucosal appearance and histology. The demographic features of the patients included in the study are presented in Table 1.

None of the patients were treated with immunosuppressive drugs or antibiotics, but two patients with CC (5 days and 4 months) and one with LC (3 weeks) were treated with budesonide for the time spans indicated prior to biopsy