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ORIGINAL ARTICLE

Microscopic Colitis Evolved Into Inflammatory Bowel Diseases Is Characterized by Increased Th1/Tc1 Cells in Colonic Mucosal Lamina Propria

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Abstract
Background An association between microscopic colitis (MC), i.e., lymphocytic colitis (LC) and collagenous colitis (CC), and inflammatory bowel diseases (IBD) has been noticed. A subset of MC cases may evolve into IBD, and IBD in remission may present as MC in a histologic pattern. Moreover, MC and IBD may coexist in different regions of the bowel. A link between MC and IBD in their pathogenesis is, therefore, suggested. Abnormal mucosal immunity is likely the key.
Methods We reviewed 2324 MC cases in Calgary over 14 years and identified 20 cases evolved into IBD (IBD transformers). 13 of them were further investigated for colonic mucosal lamina propria mononuclear cells (LPMNCs), as opposed to 22 cases whose MC resolved. On their index colonic biopsy immunohistochemistry was performed to detect major T cell subsets characterized by key cytokines and master transcription factors (IFNγ and T-bet for Th1/Tc1, GATA-3 for Th2/Tc2, IL-17 and RORc for Th17/Tc17, FoxP3 for Treg/Tcreg) as well as TNFα^{+} cells (partly representing Th1). LPMNCs positive for each marker were counted (average number per high-power field).
Results IBD transformers had increased IFNγ^{+}, T-bet^{+}, TNF-α^{+}, and GATA-3^{+} LPMNCs compared to the MC-resolved cases. The LC-to-IBD subgroup had increased IFNγ^{+} and GATA-3^{+} cells compared to the LC-resolved subgroup. The CC-to-IBD subgroup had increased T-bet^{+}, TNF-α^{+}, and GATA-3^{+} cells compared to the CC-resolved subgroup. Among MC-resolved patients, more TNF-α^{+} and RORc^{+} cells were seen in LC than in CC.
Conclusion Th1/Tc1- and TNFα-producing cells, and likely a subset of Th2/Tc2 cells as well, may be involved in the MC-to-IBD transformation.

Keywords Microscopic colitis · Lymphocytic colitis · Collagenous colitis · Inflammatory bowel disease · Lamina propria mononuclear cells · T lymphocytes · Mucosal immunity

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Introduction

Microscopic colitis (MC), including lymphocytic colitis (LC) and collagenous colitis (CC), is a peculiar chronic inactive inflammatory disease of the bowel, characterized by watery diarrhea, normal or nearly normal colonic mucosa macroscopically on routine colonoscopy, but microscopically showing increased mononuclear cells in lamina propria, lymphocytic infiltration in surface and cryptal epithelium, and/or abnormally excessive collagen deposits under the surface epithelium basement membrane

[1]. The incidence of MC varies from 4.8 to 24.8 per 100,000 persons per year in Europe and North America [1–3]. The pathogenesis of MC is yet not fully understood. Certain medications (NSAIDs and PPI), autoimmunity disorders and smoking were found to be the common contributing factors associated with the development of MC in many patients. Based on some studies, it has been believed that, ultimately, the underlying mechanism is the aberrant immune responses to luminal antigens in predisposed individuals, as evidenced by the characteristic increase in intraepithelial lymphocytes (IELs) and lamina propria lymphocytes in the involved bowel, predominantly T helper (Th) cells with mixed T helper 1 (Th1)/T cytotoxic cell (Tc1) populations and Th17/Tc17 cytokine profiles [4–10].

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), on the other hand, is a much more severe and active form of chronic inflammatory disease of the bowel, which is known to be mediated by aberrant mucosal immunity, particularly the adaptive immunity. Many studies [11–16], including our own [14, 15], have revealed an increased prevalence of Th17, Tc17, regulatory T helper cells (Treg) and regulatory cytotoxic T cells (Tcreg) in colonic lamina propria of IBD patients, as compared with healthy individuals, with CD having more Th1 and Tc1 while UC having more Th2 cells.

Therefore, both MC and IBD appear to be immune-mediated in some way. Moreover, an interesting association between MC and IBD has long been suggested in various aspects. First, evolution from MC into IBD, either UC or CD, in a small subset of MC patients has been observed clinically [17–20]. Second, on the other hand, some patients with IBD in remission or an early phase of new onset or recurrent IBD, particularly CD, may present a histologic pattern of MC [21] (unpublished personal experience as well). Third, in some patients, MC and IBD can coexist in different regions of the bowel [22–25]. Having all these taken together, it has been postulated that there should be a link between MC and IBD, and the two diseases may be the two ends of the spectrum of a chronic immune-mediated inflammatory disease of the gut, with IBD being the extreme end while MC being the mildest form.

However, the pathogenic link between MC and IBD has not been well established through systematic epidemiologic study and basic science research. Furthermore, it is not known that whether there is a specific immunologic pattern that predisposes MC to change into IBD. To identify such an immunoprofile, if it exists, would help to identify a subset of MC patients and intervene in their outcome. It would also help us get new insights into the common ground of the chronic immune-mediated inflammatory disorders of the bowel.

We are presenting a limited study with respect to the aforementioned points. In this study, we have firstly reviewed all of the patients with MC in a Canadian city (with a population over a million) over a period of 14 years, by using a city-wide centralized pathology database, and identified a subpopulation of patients who have evolved into IBD. Meanwhile, we have identified some IBD patients, within the same period of time, who presented as MC histologically when they were in remission after medical treatment. Furthermore, we have analyzed immunohistochemically certain T cell lineage profiles of cytokines and transcription factors in the lamina propria of colonic mucosa biopsied from the subset of MC patients who evolved into IBD. Additionally, we have made a comparison of the mucosa immunity between LC and CC, the two different, but sometimes interchangeable, histologic subtypes of MC.

Materials and Methods

Patient Populations and Study Design

We have carried out a computerized search of the Anatomic Pathology database of Calgary Laboratory Services (CLS), the sole pathology laboratory serving the entire Calgary region (Calgary Health Region/Alberta Health Services Calgary Zone) since 1986, to retrieve all cases of colonic biopsies upon which the diagnosis of "MC," either "LC" or "CC" or combined (i.e., both forms existed in different regions of the colon), was made histopathologically during the years 1999 to 2012 (14 consecutive years). All individual patients were thus identified based on those biopsies. The histopathology reports on all of the searchable colonic biopsies both prior and subsequent to the initial diagnosis of MC in all of the patients were thoroughly reviewed, and they were all electronically available in our system that contains the entire pathology records since the year 1980. This study was approved by the Conjoint Health Research Ethics Board of the University of Calgary.

The histopathological diagnosis of MC was made by following the universally accepted criteria [1, 26, 27]. LC is defined by increased intraepithelial lymphocytes (IELs; >20 per 100 surface epithelial cells) and mononuclear cells in lamina propria, while the cryptal architecture is preserved and the active (neutrophilic) inflammation is absent or minimal. CC is characterized by a thickened subepithelial collagen band (>10 μm) under the basement membrane, mostly accompanied by the features of LC, except for the highly increased IELs. The patient who met with the histopathological criteria of both LC and CC in separate biopsies taken from different regions (or mixed as

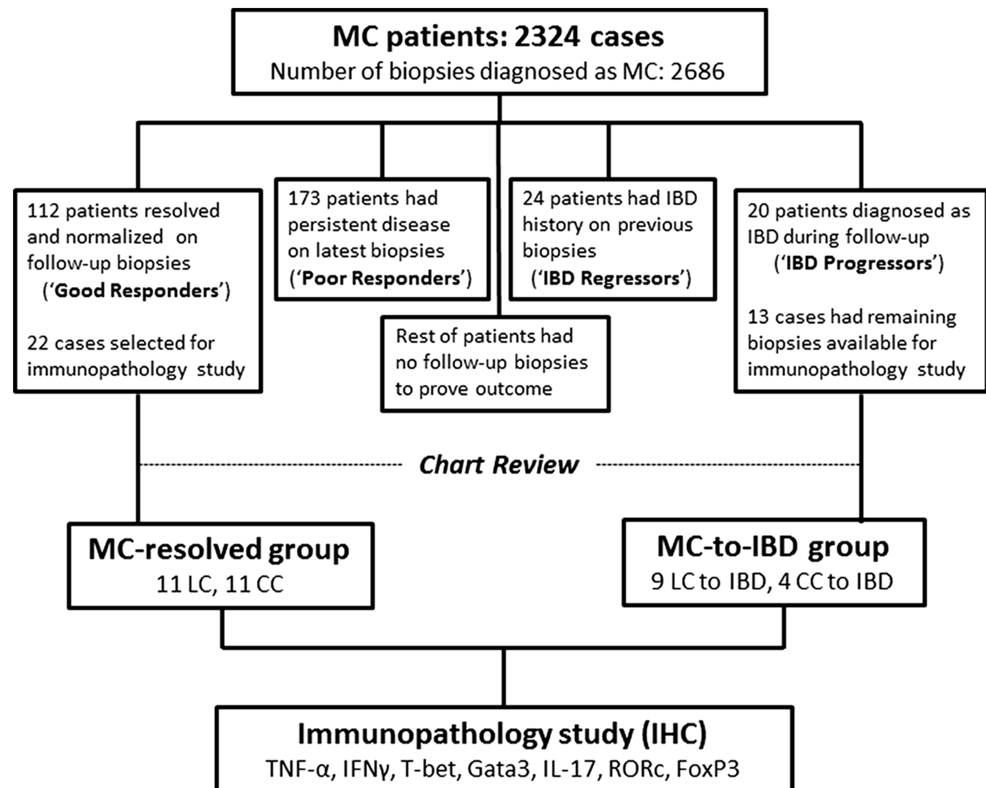


Fig. 1 Flow chart of the patient group categorization and study design

"random colonic biopsy") in a single colonoscopic procedure was defined as "combined LC and CC (LC-CC)" in this study.

Subsequently, four distinct subgroups of patients were identified in those whose outcomes were histologically proven: (1) Good responder (i.e., MC resolved and colonic mucosa normalized completely on later biopsies after standard medical treatment); (2) Poor responder (i.e., disease persisted till the latest biopsies despite of treatment); (3) IBD transformer (i.e., IBD, either UC or CD, was diagnosed on later biopsies and confirmed both clinically and endoscopically); and (4) IBD regressor (i.e., patients had history of clinico-pathologically confirmed IBD but later presented as a MC pattern in otherwise normalized colonic mucosa when IBD was in remission after treatment).

Furthermore, immunopathological study was performed in selected cases from the IBD transformer and good responder subgroups. The immunopathological study was carried out by detecting various T lymphocyte subpopulations in colonic mucosal lamina propria, which was done by using immunohistochemistry (IHC) performed on the index colorectal biopsies that yielded the initial diagnosis of MC prior to any treatment. When more than one tissue block of colonic mucosa biopsies (e.g., biopsies from different regions of colon) existed for a single patient, only one block that contains the most diagnostic and most representative biopsy was used for the IHC study.

The entire study design is summarized in Fig. 1.

Detecting T Cell Subsets by Using Immunohistochemistry

The identification of various subsets of T lymphocytes in lamina propria was defined by the immunohistochemical expression of characteristic cytokines and master transcription factors. Th1/Tc1 was defined by expression of IFNγ and T-bet, Th2/Tc2 by expression of GATA-3, Th17/Tc17 by expression of IL-17 and RORc, and Treg/Tcreg by expression of FoxP3. TNFα^{+} cells, partly representing Th1, were also separately detected.

The IHC protocol of immunohistochemistry is shown in Table 1. All antibodies used for the IHC were purchased from reputable diagnostic antibody suppliers, with their specificities having been validated. The lamina propria mononuclear cells (LPMNCs), which showed a similar cellularity/density on routine histology in all cases, and that were immunohistochemically positive for each marker were counted manually under microscope in 5 to 10 high-power fields (HPFs, ×400; depending on the size of the tissue available). The average number (per HPF) was calculated to represent the prevalence of a certain T lymphocyte subset.

Table 1 Antibodies used for immunohistochemistry

Protein	Antibody	Source	HIER (mins)	Dilution
IFN-γ	Rabbit polyclonal, clone bs-0418R	Biossusa, cat# ABIN669141	ER2 (30)	1:100
T-bet	Rabbit polyclonal, clone H-210	Santa Cruz, cat# cs-21003	ER1 (20)	1:75
GATA-3	Rabbit polyclonal	Sigma, cat# HPA029731	ER1 (30)	1:750
IL-17	Rabbit polyclonal	Sigma, cat# HPA052258	ER1 (30)	1:100
RoR-γt	Rabbit polyclonal, clone 6F3.1	Millipore, cat# MABF81	ER1 (10)	1:1000
FoxP-3	Mouse monoclonal	Abcam, cat# ab22510	ER1 (30)	1:50
TNF-α	Mouse monoclonal, clone 52B83	Abcam, cat# ab1793	ER1 (30)	1:100

Statistics

Continuous variables are shown as the mean ± SD. The probability of significant difference between two groups was analyzed using the Student's t test and chi-squared tests for continuous and categorical variables, respectively. Significance was defined as a two-sided p value <0.05. Data were collected in a Microsoft Excel database (Microsoft Excel 2010; Microsoft Corp., Seattle, WA, USA) and statistically analyzed using SPSS software for Windows, release 19.0 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 5.0 (Graphpad Inc., La Jolla, CA, USA).

Results

Classification of Cases into Different Subgroups and Demographic Data

Two thousand six hundred and eighty six colonic biopsy accessions from 2324 patients (female 1810, male 314, age 15–97, average 56 years old), all bearing the diagnosis of MC, were retrieved. Since the data was collected through a city-wide centralized healthcare record system, the number of cases ($n = 2324$) represents the entire number of cumulative newly diagnosed patients with MC over 14 years in the region with a population of roughly 1.2 million [28]; so, the prevalence was approximately 193.67 per a population of 100,000 during that period of time, and the estimated annual incidence rate was 13.83 per 100,000 person-years on average.

Upon analyzing the histologic outcomes, four particularly distinct subgroups of cases were categorized. First, 112 patients (80 LC, 30 CC, and 2 combined CC-LC) had resolved completely after medical treatment, proven by normalized colonic mucosa on later biopsy. They were classified as the "good responder" subgroup (group 1). Second, 173 patients (78 LC, 85 CC, 10 combined CC-LC) had persistent disease, despite similar medical treatments, also based on the findings in the latest follow-up biopsies. These cases were classified as the "poor responder" subgroup (group 2). Third, and most notably, by the end of year 2012, 20 patients with the initial diagnosis of MC (16 LC, 3 CC, 1 combined LC-CC) were found to have evolved into, or mixed with, IBD(12 UC, 8 CD) within this period of time, and all were confirmed histopathologically (as exampled in Fig. 2) as well as clinico-endoscopically through slides and charts review. These cases were classified as the "IBD transformer" subgroup (group 3). Of this group of cases, one developed CD 1 year after MC was resolved. In another case, MC and UC were transformed back and forth in the later course of disease over 13 years. 5 other cases showed coexisting/overlapping patterns of MC (4 LC, 1 CC) and IBD (2 CD, 3 UC) in different regions of the bowel, followed by the diagnosis of IBD being established. Additionally, in 24 cases a previous history/diagnosis (both clinical and pathological) of IBD (13 UC, 11 CD) was identified and the diagnosis of MC (17 LC, 5 CC, 2 combined CC-LC) was made on the biopsies during the post-treatment remission of IBD (with no more histologic features of active or obvious quiescent IBD). This subset of cases was classified as the "IBD regressor" subgroup (group 4). The data of all four groups of cases is summarized in Table 2. For the patients in the latter two groups (3 and 4), clinical chart review was conducted. None of the MC-to-IBD patients had any previous diagnosis and presentations of IBD, both clinico-endoscopically and histopathologically, and none of the IBD-to-MC patients had history of MC prior to the diagnosis of MC. The MC-to-IBD patients also developed bloody diarrhea and some other presentations of IBD and showed IBD-type endoscopic abnormalities at the time when the diagnoses of IBD were made. In the IBD-to-MC patients, the MC was determined based solely on the histologic pattern seen in the follow-up colonic biopsies, i.e., characteristic LC- or CC-type histologic features were seen, while characteristic features of IBD (e.g., cryptitis, crypt architecture distortion) were absent. Some of the patients had IBS-like symptoms, but none of them presented with bloody diarrhea or obvious mucosal inflammation on endoscopy.

Histologic outcome analysis also identified a two-way shift of the two subtypes/patterns of MC, LC and CC, in some patients over the course of disease, which was particularly common in group 2 patients, as shown in Table 3.

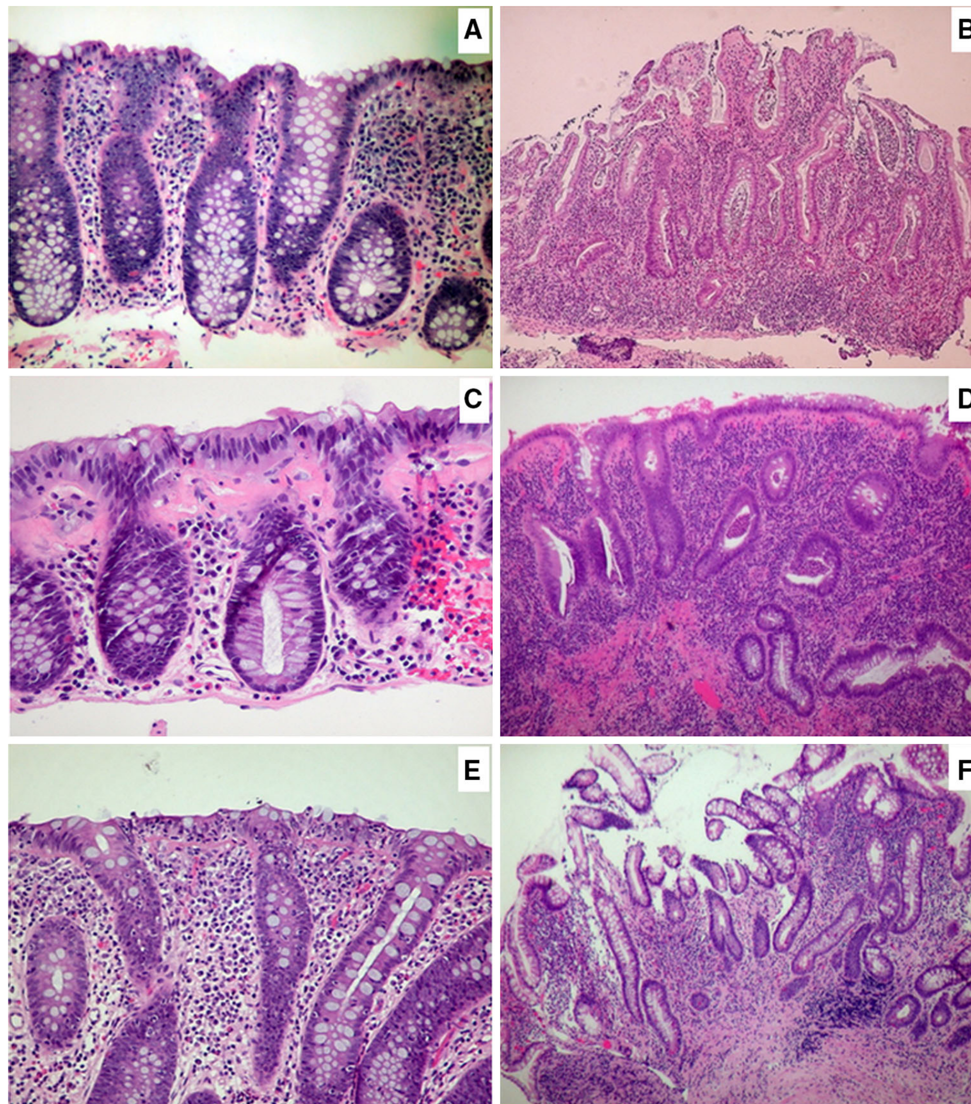


Fig. 2 Three examples of IBD progressor cases [**a**, **b** LC evolved into UC (case #5), **c**, **d** CC into UC (case #1), **e**, **f** LC into CD (case #4)]. **a** and **e** LC with increased intraepithelial lymphocytes and lamina propria mononuclear cells, and with preserved crypt architecture (×200). **b** and **d** UC with widespread crypt architecture abnormality and a diffuse transmucosal lymphocyte infiltration, neutrophilic cryptitis and cryptic abscesses (×100). **c** CC with a thick band of collagen deposits beneath the surface epithelium of colonic mucosa, accompanied by increase of lamina propria mononuclear cells (×200). **f** CD of terminal ileum (TI) with slightly patchy active ileitis with crypt architecture alteration (×100)

Further immunopathological study was performed on selected cases from the following two subgroups, after histological confirmation of diagnoses, clinical chart review, and check for tissue block availability. (A) IBD transformer subgroup (i.e., MC evolved into IBD), including 13 cases (5 LC to UC, 4 LC to CD, 4 CC to UC) from the abovementioned group 3. From that same group, five cases with mixed MC and IBD (MC/IBD overlapping) were excluded, considering that these cases may simply be a developing IBD. The remaining two cases failed to be included because their index biopsy tissue blocks were not available anymore. (B) MC-resolved group (i.e., MC without any features of, or transformation into, IBD and later resolved completely), including 22 cases (11 LC, 11 CC) semi-randomly selected from the abovementioned good responders (group 1), partly based on the availability of index biopsies, and partly to match with the other group in patients' gender and age. The detailed demographic data of the two groups of cases are summarized in Tables 4 and 5.

As shown in Table 6, there was no significant difference in gender (female predominance; 69.2 vs. 72.2%, $p = 0.292$) and the mean age at diagnosis (58.0 ± 13.2 vs. 59.7 ± 11.7, $p = 0.780$) between the IBD transformer group and MC-resolved group. The same was true when comparing the subgroup/subtypes of MC, i.e., LC-to-IBD

Table 2 Four distinct subgroups of microscopic colitis patients

Subgroup/outcome	Number of cases (M/F)	% Total cases	LC/CC/LC-CC	IBD (UC/CD)	Age (y) average (range)	Time of duration (mons) from MC to outcome average (range)
Group 1/good responder	112 (27/85)	4.82	80/30/2		59 (15–88)	41 (2–143)
Group 2/poor responder	173 (31/142)	7.44	78/85/10		65 (16–97)	48 (1–155)
Group 3/IBD transformer	20 (7/13)	0.86	16/3/1	12/8	61 (30–95)	19 (0–69)
Group 4/IBD regressor	24 (7/17)	1.03	17/5/2	13/11	55 (29–77)	69 (7–216)

LC lymphocytic colitis, *CC* collagenous colitis

Table 3 Microscopic colitis pattern/subtype shift over disease course

Pattern/subtype shift	Number of cases	% Total cases
LC → CC	27	1.16
CC → LC	21	0.90
LC ↔ CC	6	0.26
LC → LC/CC	5	0.22
CC → LC/CC	2	0.09
LC/CC → LC	6	0.26
LC/CC → CC	6	0.26

LC lymphocytic colitis, *CC* collagenous colitis, *LC/CC* combined lymphocytic and collagenous colitis in different regions of the bowel

subgroup versus LC-resolved subgroup, and CC-to-IBD subgroup versus CC-resolved subgroup.

The median (25, 75%) duration from the initial diagnosis of MC to the first diagnosis of IBD was 23 (13, 35) months. Within the IBD transformer group, 9 LC patients evolved into UC (5) or CD (4), while 4 CC patients all evolved into UC.

The entire study design is summarized in Fig. 2.

Prevalence of Different T Lymphocyte Subsets in LPMNCs

Figure 3 shows representative examples of the immunohistochemically positive LPMNCs for different markers.

As shown in Fig. 4, when comparing the cell counts (per HPF) of different T cell subsets between IBD transformer group and MC-resolved group, the IBD transformer group showed a significantly higher prevalence of IFNγ^+ cells (23.54 ± 11.71 vs. 13.22 ± 6.86, $p < 0.01$; Fig. 4a), T-bet$^+$ cells (167.41 ± 37.38 vs. 122.35 ± 48.31, $p < 0.01$; Fig. 4b), TNF-α^+ cells (25.26 ± 19.62 vs. 11.18 ± 10.85, $p = 0.01$; Fig. 4c), and GATA-3$^+$ cells (283.12 ± 75.98 vs. 219.87 ± 45.32, $p < 0.01$; Fig. 4d). There was no significant difference with regard to the subpopulations of IL-17$^+$ cells (Fig. 4e), RORc$^+$ cells (Fig. 4f), and FoxP3$^+$ cells (Fig. 4g) between these two groups.

In further analysis with regards to the subtypes of MC, the *LC-to*-IBD subgroup had increased prevalence of IFN-γ^+ cells (23.04 ± 9.15 vs. 12.59 ± 5.88, $p = 0.01$; Fig. 4a) and GATA-3$^+$ cells (275.06 ± 73.48 vs. 207.29 ± 50.10, $p < 0.05$; Fig. 4d), as compared to the LC-resolved subgroup. Similarly, the CC-to-IBD group had increased prevalence of T-bet$^+$ cells (180.26 ± 23.20 vs. 107.63 ± 33.81, $p < 0.01$; Fig. 4b), TNF-α^+ cells (20.87 ± 15.92 vs. 6.18 ± 3.32, $p < 0.01$; Fig. 4c), and GATA-3$^+$ cells (307.30 ± 94.79 vs. 231.30 ± 39.32, $p < 0.05$; Fig. 4d) as compared to the CC-resolved subgroup.

Within the IBD transformer group, there was no significant difference in the prevalence of any of the lymphocyte subsets between the two subtypes of MC (LC-to-IBD versus CC-to-IBD; Fig. 4a–g).

With regard to the comparison between MC-to-UC ($n = 9$) and MC-to-CD ($n = 4$) cases, based on the small number of cases, the only difference was seen in the number of T-bet$^+$ cells, which was significantly higher in MC-to-CD cases than that in MC-to-UC cases (198.16 ± 32.40 vs. 153.74 ± 31.92, $p = 0.042$; Fig. 4h), while the other T cell subsets showed no significant difference. In further analysis on comparing between LC-to-UC ($n = 5$) and LC-to-CD ($n = 4$) cases, based on the small number of cases, the only difference was seen in the number of T-bet$^+$ cells, which was significantly higher in LC-to-CD cases than that in LC-to-UC cases (198.16 ± 32.40 vs. 132.52 ± 19.20, $p = 0.007$), while the other T cell subsets showed no significant difference.

Among the MC-resolved patients, LC-resolved subgroup had a higher prevalence of TNF-α^+ cells (16.18 ± 13.45 vs. 6.18 ± 3.32, $p < 0.05$; Fig. 4c) and RORc$^+$ cells (15.39 ± 5.49 vs. 9.13 ± 4.52, $p = 0.01$; Fig. 4f) than that in the CC-resolved subgroup.

Discussion

The phenomenon of MC and IBD being associated with and/or transformed into each other in a subset of patients, either MC evolving into IBD or healing IBD presenting as MC, has been recognized in clinical practice [17–19, 25],

Table 4 Demographic data of 13 patients who evolved into IBD

Case#	Sex	Age at 1st diagnosis	Initial diagnosis of MC		Region of biopsy for IHC	Later diagnosis of IBD		Interval between MC to IBD (mons)	Back to MC	History prior to MC
			Type	Bowel region		Type	Bowel region		Y or N/type	
1*	F	60	CC	AC/TVC/DC/SC	DC	UC	PC	32	Y/CC	Pseudomembranous colitis
2	M	72	LC	SC	SC	UC	SC/PC	19	N	Reflux esophagitis
3	F	39	LC	TVC	TVC	UC	TVC/DC/SIG/REC	35	N	Unknown
4	F	60	LC	AC/REC	AC	CD	TI	11	N	Skin SCC
5	M	26	LC	CE/AC/DC	AC	UC	REC	9	N	None
6	M	33	LC	PC (random Bx)	Random Bx	UC	PC	52	N	PSC
7	F	75	CC	PC (random Bx)	Random Bx	UC	PC	35	N	Unknown
8	M	79	LC	PC (random Bx)	Random Bx	UC	PC	16	N	Colonic adenomatous polyps
9	F	71	CC-LC	CE/TVC/SC/REC	SC (CC)	UC	CE/Rt	83	N	H. pylori-negative chronic gastritis
10	F	55	LC	Rt	Rt	CD	TI/Rt	23	N	H. pylori-gastritis
11	F	75	CC	PC	Lt	UC	PC	30	N	Unknown
12	F	54	LC	Rt	Rt	CD	TI	23	N	H. pylori-negative chronic gastritis
13	F	55	LC	Rt	Rt	CD	TI	13	N	Cholelithiasis

MC microscopic colitis, *LC* lymphocytic colitis, *CC* collagenous colitis, *UC* ulcerative colitis, *CD* Crohn's disease, *PC* pan colon, *CE* cecum, *AC* ascending colon, *TVC* transverse colon, *DC* descending colon, *SC* sigmoid colon, *REC* rectum, *Rt.* right colon, *Lt.* left colon, *TI* terminal ileum, *Bx* biopsy

Table 5 Demographic data of 22 patients with resolved non-IBD-associated MC

Case#	Sex	Age	MC			Region of Bx for IHC	Interval prior to normalized bx (mons)	History prior to MC
			Type	Bowel region	Type shift			
1	F	58	LC	PC	No	Random Bx	24	Unknown
2	F	39	LC	PC	No	Random Bx	65	Reactive gastropathy
3	F	72	LC	PC	No	Random Bx	26	Candida esophagitis
4	M	54	LC	PC	No	Random Bx	35	Unknown
5	M	47	LC	PC	No	Random Bx	51	Chronic hepatitis C
6	F	55	LC	PC	No	Random Bx	26	Barreett's esophagus
7	F	48	LC	PC	No	Random Bx	28	Unknown
8	F	34	LC	PC	No	Random Bx	29	NASH, cholelithiasis
9	M	54	LC	PC	No	Random Bx	23	Chronic hepatitis C
10	M	54	LC	PC	No	Random Bx	12	Colorectal adenomatous polyps, peptic duodenitis
11	F	74	LC	PC	No	Random Bx	32	Celiac disease
12	F	54	CC	PC	No	Random Bx	19	Barreett's esophagus, nodular goiter of thyroid
13	F	78	CC	PC	No	Random Bx	56	Unknown
14	F	60	CC	Rectosigmoid	No	Rectosigmoid	56	Breast fibroadenoma
15	F	54	CC	PC	No	Random Bx	63	Celiac disease
16	F	74	CC	AC/DC/SC/REC	Yes/LC	AC	53	Unknown
17	F	54	CC	PC	No	Random Bx	69	Hysterectomy for adenomyosis, skin melanoma and basal cell carcinoma
18	M	64	CC	DC/SC/REC	Yes/LC	DC	16	Acute appendicitis, skin basal cell carcinoma, cholelithiasis, cholecystitis
19	F	75	CC	PC	No	DC	73	Cholelithiasis, cholecystitis
20	F	40	CC	PC	No	Rt.	44	CC with pseudomembranous features
21	F	62	CC	PC	No	Rt.	77	Unknown
22	F	63	CC	PC	No	Rt.	37	Parathyroid adenoma

MC microscopic colitis, *LC* lymphocytic colitis, *CC* collagenous colitis, *UC* ulcerative colitis, *CD* Crohn's disease, *PC* pan colon, *CE* cecum, *AC* ascending colon, *TVC* transverse colon, *DC* descending colon, *SC* sigmoid colon, *REC* rectum, *Rt.* right colon, *Lt.* left colon, *TI* terminal ileum, *Bx* biopsy

Table 6 Comparison of demographic characters between different groups

Groups	Case number	Gender			Age	
		Male (%)	Female (%)	*p* value	Mean ± SD	*p* value
MC-to-IBD	13	4 (30.8)	9 (69.2)	0.292	58.0 ± 13.2	0.768
MC-resolved	22	6 (27.8)	16 (72.2)		59.7 ± 11.7	
LC-to-IBD	9	4 (44.4)	5 (55.6)	1.000	52.6 ± 13.3	0.806
LC-resolved	11	11 (36.4)	7 (63.6)		54.3 ± 11.3	
CC-to-IBD	4	2 (50.0)	2 (50.0)	0.516	70.3 ± 5.1	0.326
CC-resolved	11	2 (18.8)	9 (81.2)		65.1 ± 9.8	

MC microscopic colitis, *LC* lymphocytic colitis, *CC* collagenous colitis

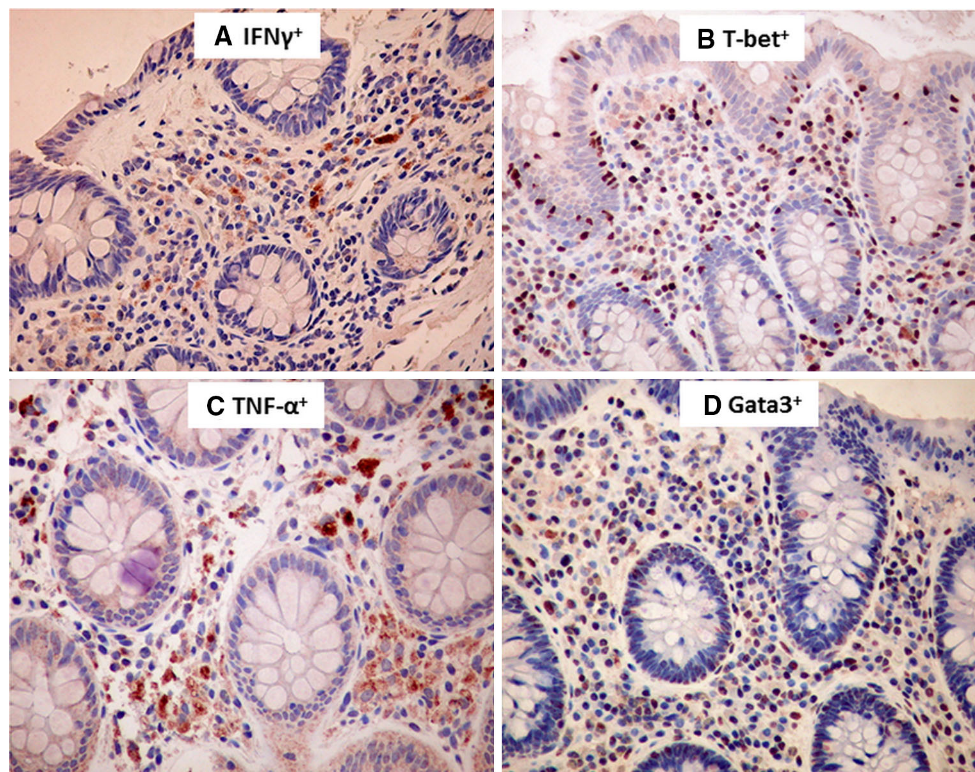
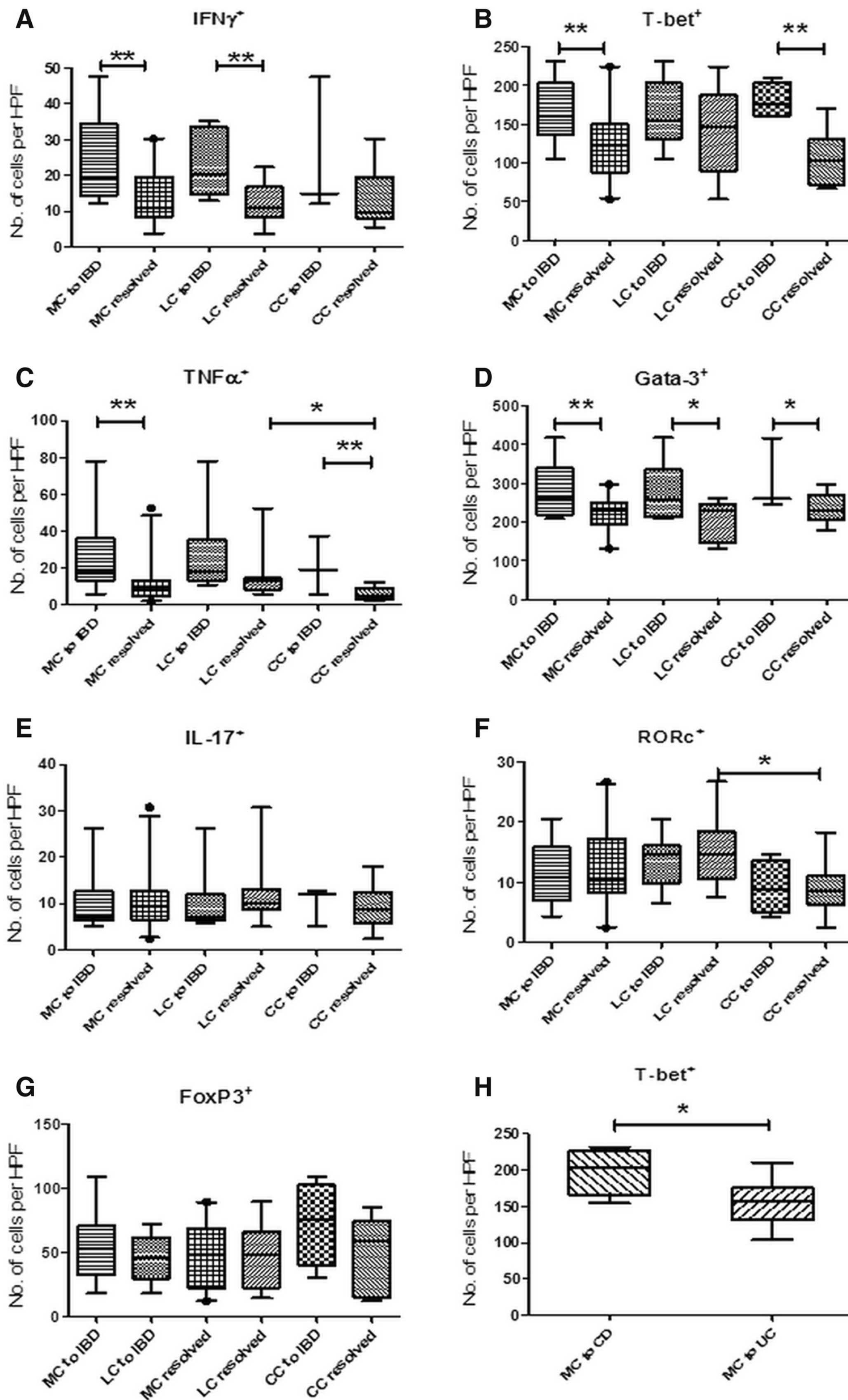


Fig. 3 Representative graphs of immunohistochemical staining for IFNγ (**a**), T-bet (**b**), TNFα (**c**) and GATA-3 (**d**) ×400. Immunopositive lymphocytes in lamina propria of colonic mucosa

which raises a question about the underlying mechanism that links these two entities. Some investigators have even considered MC as a "gentler form of IBD" [29]. It seems to be most reasonable to postulate that the link occurs in the changes of mucosal immunity with a common aberrant adaptive immunity shared by the two conditions. The patients who develop MC and later evolve into IBD may have some distinctively characteristic features as compared with those whose MC responds well to the current medical treatment (i.e., good responders) and never change into IBD.

As presented here, we have conducted a population-based retrospective and systematic study in a medium-sized Canadian city where the centralized healthcare system enabled us to follow up the patients' outcome through the longitudinal medical records. Three major and novel findings were yielded. First, the prevalence of IBD evolvement/transformation in patients with MC was nearly 1%. Second, the subset of MC that evolved into IBD had higher prevalence of Th1/Tc1, Th2/Tc2 and TNF-α^+ lymphocyte subsets in lamina propria, as compared with those who completely resolved. Last, among the MC cases that resolved completely, there were more TNF-α^+ and RORc$^+$ LPMNCs in LC than that in CC.

Scattered case reports of MC "transformed/progressed" to IBD have been seen in literature, including LC to UC,



A
IFNγ+
Nb. of cells per HPF
**
**
MC to IBD
MC resolved
LC to IBD
LC resolved
CC to IBD
CC resolved
B
T-bet+
**
**
C
TNFα+
**
*
**
D
Gata-3+
**
*
*
E
IL-17+
F
RORc+
*
G
FoxP3+
H
T-bet+
*
MC to CD
MC to UC

◀**Fig. 4** Prevalence of different T lymphocyte subsets in lamina propria mononuclear cells (LPMNCs) among the different groups. The height of bars represents the average count of positive cells per high-power field (HPF). (*$p < 0.05$, **$p < 0.01$)

CC to UC, LC to CD, and CC to CD [17–19, 22, 30]. The reported incidence of IBD seen in MC patients was 0–2.5% [22]. On the other hand, some cases of IBD "converted" to or complicated with MC, including UC converted to CC and CD complicated with CC [21, 24, 31]. In our data, we have encountered 20 (0.86% of total) MC patients evolving into IBD, and 29 (1.25% of total) MC patients having previous history of IBD, which shows incidences similar to that reported before. In our cases that were classified as IBD transformers, none of them had previous history of established classic IBD prior to the diagnosis of MC, and their later diseases presented as, and followed a course of, typical IBD but not merely IBD-like features superimposed to MC as described in some studies [29]. All of these observations not only suggest an association between MC and IBD but also indicate that MC can, in fact, be an atypical histologic pattern as the prodrome and/or show-down signs in the course of IBD during its early development and post-therapy remission stages. It is also possible that rare cases of those MC-to-IBD transformers were IBD in nature in the first place but simply started with an atypical form in histology, or occasional cases just developed IBD independently over a background of MC. Since the majority of MC patients do not develop IBD and not all IBD patients would change to MC, the association between the two only exists in a small subset of patients in whom the pathogenic mechanisms may deviate from general mechanisms.

Aberrance of gut immunity, both adaptive and innate immune systems, especially the adaptive immunity in lamina propria, has been considered to be the key mechanism in the pathogenesis of both IBD and MC [33, 34]. With respect to the details of immunity disorder in MC, the studies are limited so far, and the data from different studies were not entirely consistent and even contradictory. Phenotypically, MC is characterized by increase of IELs and LPMNCs [32, 35]. The IELs are typically $CD8^{+}CD4^{-}$ T-cytotoxic cells, while the T lymphocytes in lamina propria are predominantly $CD8^{-}CD4^{+}$ T-helper cells [7, 8, 36]. Furthermore, in MC, a mixed Th1/Tc1 and Th17/Tc17 mucosal cytokine profile has been revealed by a few studies, including our own [5, 6, 37]. Overexpression of mRNA of IFNγ and TNFα, both Th1/Tc1linage cytokines, was also a common finding seen in both LC and CC. Kumawat et al. [5] also showed increased mRNA expression of multiple Th17/Tc17 lineage cytokines including IL-1β, IL-6, IL-17, IL-21 and IL-22. The mRNA level of RORC, the master transcription factor of Th17/Tc17 cells, was found not different between MC and controls, the same as the mRNA levels of Th2 lineage cytokines IL-5 and IL-10. Moreover, the protein levels of the aforementioned cytokines, except IL-21, IL-6 and IL-22, failed to show a difference between MC and controls. Additionally, Fernández-Beñares et al. [38] showed an increase of $CD25^{+-}$ $FoxP3^{+}$ Treg cell population in LPMNCs. Jöhrens et al. [7] found that the expression of GATA-3, the key transcription factor of Th2 cells, was detected in up to 30–40% of the lamina propria T cells in LC, while T-bet, the key transcription factor of Th1 cells, was also expressed in 30–50% of the T cells. So, they proposed that LC represented a combined Th1/Th2 response caused either by an intra- or extra-cellular microorganism or by a hypersensitivity reaction to luminal antigens. Here, we have also detected all of Th1, Th2, Th17, and Treg cells in colonic mucosal LPMNCs in the setting of MC, in which GATA-3^{+} Th2 and T-bet^{+} Th1 cells comprising the two predominant populations in terms of their cell counts. The findings are in agreement with the previous studies.

More importantly, in the present study, we have found that MC patients with different outcome appeared to have different features of mucosa immunity. The IBD transformers had further higher prevalence of Th1/Tc1 and Th2/Tc2 cells in colonic LPMNCs. Although one may argue that the difference may simply because of denser lymphocytes in lamina propria in the IBD transformers, it was not our impression under routine histological examination. On the other hand, there was no difference in Th17/Tc17 and Treg/Tcreg lymphocytes between the two groups. The increased ratio of T effector lymphocytes over Treg lymphocytes seems to be the key characteristic feature of mucosa immunity in MC that would transform to IBD. Moreover, a further increased number of T-bet^{+} Th1 cells was seen in the patients who transformed into CD than those into UC. Although the latter result may not be sufficiently convincing, in consideration of the small number of cases, the finding is partly in agreement with our previous findings in an IBD study in which a higher prevalence of T-bet^{+} cells was seen in CD than that in UC patients [15]. Currently, the distinct immunity changes that differentiate MC from UC and from CD have not been specified. It is not possible for us to identify or propose the factors that may trigger a transformation from MC to IBD. For this purpose, comparative and expanded studies, including separation of T helper and T cytotoxic cells, would be needed.

The two subtypes of MC, LC, and CC, although considered two separate entities, actually share most of the histologic features and differ only by whether or not there is a subepithelial collagen deposition [8, 31]. The incidences of both entities are also approximately parallel, and their clinical presentation and response to therapy are

almost identical. Moreover, in many patients, a shift from one to the other during the course of disease is not uncommon [39], as demonstrated in the present data as well. All of these indicate that CC and LC are simply different histological patterns of the same disease [8]. The difference in their pathogenesis, however, is not well understood. In limited studies, the mucosal immunity in the LC and CC was found to be not entirely the same. Bai et al. demonstrated more $CD8^+$ but less $FoxP3^+$T cells in lamina propria in LC than that in CC [40]. Our data showed that among the MC-resolved patients, those with LC had a higher prevalence of TNF-α^+ cells and $RORc^+$ cells in lamina propria than those with CC.

To our knowledge, this study is the first one that focuses on characterizing the differences in mucosa immunity between IBD transformers and MC-resolved groups. Several limitations of this study, however, need to be mentioned. First, due to the rarity of cases with MC evolving into IBD, the mucosal immunity study using IHC was only conducted in limited selected cases and not the entire patient population. Second, no healthy controls (HCs) and active IBD patients were enrolled at the same time, which made it impossible to systemically and simultaneously analyze and compare the mucosa immunity changes in MC, IBD, and HC settings, although to show such differences was not the purpose of this study. Third, single-IHC staining for each of the T lymphocyte lineage characteristic cytokines and transcription factors individually is a less ideal protocol than using double- or triple-IHC staining for multiple markers simultaneously. Finally, the test panel for cytokines and T cell subpopulations was far from comprehensive and, therefore, undoubtedly failed to reveal more possible changes. A multicenter and prospective study using double- or triple-IHC staining or even flow cytometry on fresh mucosal samples and using an expanded panel of markers would be more ideal and needed in the future studies.

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Compliance with ethical standards

Conflict of interest None of the authors has any conflicts of interest.

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