Colonic Phenotype of the Ileum in Crohn's Disease: A Prospective Study Before and After Ileocolonic Resection

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Background: Colonic metaplasia has been described in pouchitis. In a prospective study, we investigated whether colonic phenotype may develop in Crohn's disease (CD) ileum. The expression of sulfomucins (colonic mucin), sialomucins, and CD10 (small intestine mucin and phenotype) was evaluated before and after ileocolonic resection for CD.

Methods: From February 2007 to March 2010, 22 patients with CD undergoing surgery were enrolled. Clinical (Crohn's Disease Activity Index >150) and endoscopic recurrence (Rutgeerts score ≥1) rates were assessed at 6 and 12 months. Ileal samples were taken at surgery (T0), at 6 (T1), and 12 months (T2) for histology, histochemistry (High Iron Diamine-Alcian Blue), and immunohistochemistry (anti-CD10).

Results: In 22 patients, recurrence was assessed at 6 and 12 months (clinical recurrence 9% and 18%; endoscopic recurrence 73% and 77%). In all 22 patients, ileal samples were taken at 6 and 12 months (involved area in patients with recurrence). In 19 of 22 (86.3%) patients, the involved ileum was also studied at surgery. At T0, T1, and T2, the expression of sialomucins and CD10 (small intestine mucin and phenotype) was comparable and higher ($P < 0.0001$) than the expression of sulfomucins (colonic mucin) (mean [range], T0:82 [35–100] versus 75 [0–100] versus 16 [0–50]; T1:96 [60–100] versus 94.7 [50–100] versus 3.89 [0–40]; T2:93.3 [60–100] versus 88.1 [25–100] versus 6.6 [0–40]). The expression of small-intestine mucin and phenotype was higher at T1 ($P = 0.025$) versus T0 ($P = 0.026$). Differently, the expression of colonic mucin was lower at T1 versus T0 ($P = 0.027$).

Conclusions: In CD, the ileum involved by severe/established lesions develops a "metaplastic" colonic mucosa phenotype. Differently, CD ileum with no lesions or with early recurrence maintains the "native" small intestine type mucin secretion and phenotype.

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Key Words: Crohn's disease, postoperative recurrence, ileum, colonic metaplasia

Postoperative recurrence after "curative" resection is a feature of Crohn's disease (CD).[1–4] Ileocolonoscopy is the gold standard for assessing CD recurrence after ileocolonic resection,[2,5,6] although alternative noninvasive techniques have been proposed.[7,8] At 1 year, endoscopic recurrence is observed in 65% to 80% of patients, and almost 10% of patients require multiple resections.[1–6,9–11] Consistent evidences support that the severity of the endoscopic recurrence at 1 year is predictive of clinical relapse at 3 years.[2,6] Nevertheless, the pathogenetic mechanisms of recurrence and the possible changes of the ileum after ileocolonic resection for CD are not defined. A role for luminal antigens, including the resident bacterial flora, has been suggested.[12,13] This hypothesis is also supported by the observation that fecal diversion prevents CD recurrence and that infusion of intestinal contents in excluded ileum induces early CD recurrence[14]. Changes of the bacterial flora after proctocolectomy with ileopouch anal anastomosis for ulcerative colitis (UC) have been proposed also in the pathogenesis of pouchitis.[15–18] The reported efficacy of probiotic[19–21] and antibiotics preparations[22] in pouchitis supports this hypothesis. Changes of the ileum towards colonic epithelium (colonic metaplasia) have been reported in pouchitis.[23–25] Colonic metaplasia of the ileum is characterized by villous atrophy and crypt hyperplasia with the presence of goblet cells.[25] The expression of colonic epithelial antigens (human tropomyosin isoform 5) and mucins (sulfomucins) has been described in the epithelium lining the ileal pouch.[24–27] A possible relation between development of colonic metaplasia and chronic inflammation in pouchitis has been suggested.[24–27] In a preliminary observation, we reported the expression of colonic antigens in the ileum above ileorectal anastomosis for CD.[28] The possible development of colonic metaplasia in the neoterminal ileum above ileocolonic anastomosis and its possible relation with recurrence has not been investigated.

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These observations prompted us to assess, in a prospective study, whether colonic metaplasia may be observed in CD ileum. At this purpose, in a cohort of patients with CD undergoing ileocolonic resection, the expression of both colonic mucin type (sulfomucins) and small intestine mucin type (sialomucins) and phenotype (CD10 metal protease)[29] was evaluated in ileal samples taken at surgery and during ileocolonoscopy at 6 and 12 months. Clinical and endoscopic recurrence was also evaluated.

MATERIALS AND METHODS

Study Protocol

From February 2007 to March 2010, all consecutive patients undergoing ileocolonic resection for CD with indication made according to current guidelines were enrolled.[30]

Patients

Inclusion criteria: (1) diagnosis of CD according to conventional criteria[30]; (2) regular follow-up; (3) indication for ileocolonic resection; (4) surgical resection with no residual lesions, performed by the same surgical unit; and (5) age 18- to 60-year old. Exclusion criteria: (1) low compliance; (2) Severe comorbidities; (3) incomplete colonoscopy; (4) early postoperative complications (<1 mo); and (5) pregnancy.

Clinical Assessment

In each patient, clinical details including medical treatments and comorbidities after surgery were prospectively collected in clinical records. Clinical assessment was made according to the CD Activity Index (CDAI >150)[31] at 3, 6, 9, and 12 months.

Ileocolonoscopy

In all 22 patients, endoscopic recurrence was assessed by ileocolonoscopy at 6 and 12 months, with multiple biopsies (≥2) taken from the neoterminal ileum visualized for ≥10 cm. All colonoscopies were performed by the same IBD-dedicated gastroenterologist, and findings documented by photographic verification. The severity of recurrence was assessed according to Rutgeerts et al[1] (score 0–4; recurrence: score ≥1).[4]

Histology Study

Tissue Samples

In all 22 patients, multiple ileal biopsy samples (≥2) were taken from the neoterminal ileum during ileocolonoscopy at 6 (T1) and 12 months (T2). At 6 and 12 months, biopsies were taken from the involved neoterminal ileum in patients with recurrence and from the uninvolved ileum in patients with no recurrence. In 19 of the 22 patients, evaluable surgical specimens were taken from the involved ileum (T0).

Histology Assessment

Surgical and endoscopic samples were fixed in buffered formalin (10%) and embedded in paraffin. Multiple sections (5-μm thick) were serially obtained from each paraffin block and stained with hematoxylin and eosin (H&E). The histology assessment was consistent with the conventional criteria.[1–3,6]

Histochemistry and Immunohistochemistry Study

The mucins' histochemical profile was assessed by High Iron Diamine–Alcian blue stain (HID-AB), distinguishing sialomucins from sulfomucins.[32] The prevalence of sialo- and sulfo-mucins was assessed as percentage of sialomucins versus sulfomucins in each of the biopsy samples. All cases were jointly assessed by 2 trained pathologists with elective experience in the field. The immunohistochemistry (IHC) expression of CD10[29] was assessed on serial sections of the same tissue samples considered for the histology and histochemistry (HC) study. The target antigen was retrieved (citrate buffer, pH 6) in dewaxed histology sections. Immunostaining was done by applying anti-CD10 Rabbit Monoclonal Antibody (clone EP195, 1:50; Diagnostic BioSystems, Pleasanton, CA); the color was developed by applying the DAB/Ni peroxidase substrate kit (Vector Laboratories, Burlingame, CA). CD10 expression was arbitrarily assessed distinguishing in 4 classes as percentage of the positive immunoreaction (absence of any immunostaining or inconsistent immunostaining in <3% of the cell population [scored as 0%]; positive immunostaining in 1/4 of the cell population [scored as 25%]; positive immunostaining featured by one half of the cell population [scored as 50%]; diffuse positive stain in the large majority of the epithelial cell population [scored as 100%]). In all patients, both the HC (mucins) and IHC (CD10) profiles were assessed in the surgical tissue specimens at the time of resection (T0) and in the biopsies at 6 (T1) and 12 months (T2) after surgery.

Small Intestine Contrast Ultrasonography

In a subgroup analysis, the extent of recurrence was further assessed by small intestine contrast ultrasonography (SICUS), performed by an experienced gastroenterologist.[7,8] SICUS findings compatible with recurrence included an increased bowel wall thickness (≥3 mm) as reported.[7,8]

Statistical Analysis

Results were expressed as mean (range). Differences between groups were analyzed by the Student's t or the χ^2 test, as appropriate. Outcomes considered: (1) clinical recurrence at T1 and T2[31]; (2) endoscopic recurrence at T1 and T2; (3) percentage of expression of sulfomucins, sialomucins and CD10 in ileal samples at T0, T1, and T2. Correlation between endoscopic score and clinical activity[31] were estimated by the Spearman (r) method.

RESULTS

Patients

During the study period, 37 patients with CD undergoing ileocolonic resection were enrolled. In all patients, histological analysis of the surgical specimen confirmed the diagnosis of CD and the absence of residual lesions. Among these 37 patients, 15 (40%) were

excluded due to pregnancy (n = 1), no compliance (n = 9), incomplete colonoscopy (n = 2), severe clinical relapse (n = 1), and lost to follow-up (n = 2). The analysis therefore included 22 of the 37 patients (59%) enrolled. In all 22 patients, ileal samples were taken from the neoterminal ileum at both 6 and 12 months. In 19 of these 22 patients (86.3%), ileal samples were taken not only during ileocolonoscopy at 6 and 12 months, but also from the involved surgical specimen. Colonic metaplasia was therefore searched in 22 patients at 6 and 12 months, and in 19 of these 22 patients, not only at 6 and 12 months, but also at surgery. In 3 of the 22 patients, HC and IHC analysis indeed included only ileal samples taken at 6 and 12 months, while the surgical specimen was not evaluable. Demographics and clinical characteristics of each of the 22 patients are summarized in Table 1 (age 38.2 years [17–57]; age at diagnosis, 30.1 years [14–44]; CD duration, 8.2 [1–30]). At surgery, lesions involved the ileum in 14 (63.6%) and the ileum–colon in 8 patients (36.4%). Family history of IBD (first-degree relatives) was reported by 6 patients (27%).

Clinical Recurrence

All 22 patients were treated with mesalazine (2.4 g/d) early after surgery (<3 mo). Relapse (CDAI >150) was observed in 2 patients (9%) at T1 and in 4 patients (18%) at T2 (Table 1). These patients were treated with mesalazine and rifaximine (n = 1), budesonide (n = 1), corticosteroids and anti-TNFs (n = 1), or corticosteroids (n = 1). All the 18 inactive patients at T2 received mesalazine, with combined azathioprine in 2 patients. The mean CDAI did not significantly increase at 6 versus 12 months (90 [14–304] versus 105.5 [30–353]; $P = 0.36$).

When the analysis was restricted to the 19 patients with ileal samples taken not only at T1 and T2, but also at T0, relapse was observed in 2 patients (10.5%) at T1 and in 4 patients (21%) at T2. Inactive patients at T2 (n = 15) received mesalazine, with combined azathioprine in 3. Patients developing recurrence ≤12 months (n = 4) were also treated with corticosteroids (n = 1), anti-TNFs (n = 1), rifaximine (n = 1), or combined azathioprine and budesonide (n = 1). The mean CDAI was comparable at 6 versus 12 months (91 [14–304] versus 108 [34–353]; $P = 0.40$).

Endoscopic Recurrence

When considering the whole group of 22 patients, endoscopic recurrence (score ≥1) at T1 was observed in 16 patients

TABLE 1. Demographic, Clinical, and Endoscopic Recurrence in Each of the 22 Patients with CD Studied at Surgery at 6 and 12 months After Ileocolonic Resection

					Clinical Recurrence (CDAI >150)		Endoscopic Recurrence (Rutgeerts' score ≥1)	
Pt	Sex	Age, yr	Indication for Surgery	CD Duration, yr	6 mo	12 mo	6 mo	12 mo
1	M	37	Subocclusions	4	134	127	2	4
2	F	17	Abscess	3	14	138	2	2
3	M	38	Subocclusions	6	46	62	3	3
4	M	41	Subocclusions	17	76	121	2	3
5	F	51	Subocclusions	12	20	34	0	2
6	M	54	Subocclusions	2	119	60	2	3
7	F	57	Subocclusions	30	58	40	1	1
8	M	28	Subocclusions	1	56	56	3	3
9	M	51	Subocclusions	9	87	50	2	1
10	F	26	Subocclusions	5	72	353[a]	2	1
11	M	41	Subocclusions	1	304[a]	212[a]	3	0
12	M	43	Subocclusions	11	123	114	0	0
13	M	33	Abscess	3	148	124	4	4
14	M	44	Subocclusions	0	102	235[a]	0	0
15	F	46	Subocclusions	10	24	38	0	2
16	M	48	Subocclusions	4	193[a]	153[a]	4	4
17	M	21	Subocclusions	0	38	30	2	3
18	F	42	Subocclusions	12	68	32	0	0
19	M	33	Subocclusions	7	60	75	2	2
20	F	27	Subocclusions	5	74	118	0	0
21	M	30	Abscess	11	62	88	3	4
22	M	38	Abscess	27	103	61	4	4

[a]Patients with clinical relapse (CDAI>150).
Pt, Patient.

(72.7%) (2 showing clinical recurrence). The endoscopy scores were: grade 0 (n = 6); 1 (n = 1); 2 (n = 8); 3 (n = 4); and 4 (n = 3; with stenosis in 2) (Table 1). Both patients showing clinical relapse at T1 also showed endoscopic recurrence (scores 3 and 4). At T2, endoscopic recurrence was detected in 17 patients (77.3%). The endoscopy scores were: grade 0 (n = 5); 1 (n = 3); 2 (n = 4); 3 (n = 5); and 4 (n = 5, stenosis in 2) (Table 1). The endoscopy score did not increase at T1 versus T2 (1.86 [0–4] versus 1.79 [0–4]; $P = 0.34$). At both 6 and 12 months, there was no correlation between the CDAI, and the endoscopy scores (r = 0.37; $P = 0.08$ and r = 0.25; $P = 0.23$, respectively).

When considering the subgroup of 19 patients with evaluable surgical samples, recurrence at T1 occurred in 14 patients (73.6%). The endoscopy scores were: grade 0 (n = 5), 1 (n = 1), 2 (n = 8), 3 (n = 3), or 4 (n = 2; stenosis in 1). At T2, endoscopic recurrence occurred in 15 patients (78.9%), graded as: 0 (n = 4), 1 (n = 3), 2 (n = 4), 3 (n = 5), and 4 (n = 3; stenosis in 2). The endoscopy scores did not increase at 6 versus 12 months (1.78 [0–4] versus 2 [0–4]; $P = 0.36$).

SICUS Findings

To further assess the extent of recurrence, 17 of the 22 (77.2%) patients had also SICUS performed ≤1 year after surgery. SICUS detected findings compatible with recurrence in 12 of 17 (70.5%) patients. Ileocolonoscopy showed a grade 2 recurrence in 2 of the 5 patients showing a normal bowel wall thickness. Anastomotic CD recurrence (grade 2) may indeed be not detected by SICUS, visualizing the extraluminal, but not the intraluminal surface.[7,8]

HC and IHC Analysis

Comparison Between the Expression of Sialomucins, Sulfomucins, and CD10

Table 2 summarizes HC and IHC findings from the 22 patients studied at 6 and 12 months. At 6 months, the mean expression of sialomucins, sulfomucins, and CD10 was 95.72%, 4.27%, and 92%, respectively. At 6 months, the mean expression of sialomucins and CD10 (small intestine type and phenotype) was comparable ($P = 0.47$) and significantly higher than the expression of sulfomucins (colonic mucin-type) ($P < 0.0001$ both) (Table 2).

In the ileal samples taken from the same 22 patients at 12 months, the mean expression of sialomucins, sulfomucin, and CD10 was 93.6%, 6.4%, and 89.7%, respectively (Table 2). As observed at 6 months, at 12 months also the mean expression of sialomucins and CD10 (small intestinal phenotypes) was comparable ($P = 0.28$) and higher than the expression of sulfomucins (colonic mucin type) ($P < 0.0001$ both). Figure 1A–F shows the endoscopic view, HI, and HIC analysis of the neoterminal ileum from 1 patient with CD studied at 6 and 12 months.

To compare findings from HC and IHC analysis at surgery versus 6 and 12 months, in a subgroup analysis, only 19 of the 22 patients (85%) with ileal samples taken also at surgery were considered (Table 2). In 3 of the 22 patients, HC and IHC analysis indeed included only biopsy specimens taken at 6 and 12 months, although the surgical specimen was not evaluable. In the ileal samples taken at surgery from these 19 patients, the mean expression of sialomucins, sulfomucins, and CD10 was 82.2%, 16.2%, and 75%, respectively (Table 2). At surgery, the mean expression of sialomucins was higher than the expression of sulfomucins

TABLE 2. Expression of Sialomucins (Small Intestine Mucin Type), Sulfomucins (Colonic Mucin Type), CD10 (Small Intestine Phenotype) in ileal Samples Taken from Patients with CD at Surgery (n = 19) and During ileocolonoscopy at 6 mo (n = 22) and 12 mo (n = 22)

	Surgery (n = 19)	6 mo (n = 22)	12 mo (n = 22)
Sialomucins	82.2[a] (35–100)	95.72[b] (60–100)	93.6[c] (65–100)
Sulfomucins	16.2 (0–50)	4.27 (0–40)	6.4 (0–40)
CD10	75[a] (0–100)	92[b] (25–100)	89.7[c] (25–100)

		6 mo		12 mo	
	Surgery	Recurrence[d] (n = 16)	No Recurrence (n = 6)	Recurrence[d] (n = 17)	No Recurrence (n = 5)
Sialomucins	82.2[a] (35–100)	94.6[b] (60–100)	99[b] (95–100)	93[c] (65–100)	95[c] (80–100)
Sulfomucins	16.2 (0–50)	5.3 (0–40)	1.3 (0–5)	6.47 (0–40)	4.2 (0–20)
CD10	75[a] (0–100)	96[b] (50–100)	79.16[b] (25–100)	86.7[c] (25–100)	100[c] (100–100)

Results (mean % and range) subgrouped according to endoscopic recurrence are also included.
[a]$P < 0.0001$ expression of sialomucins and CD10 versus sulfomucins at surgery.
[b]$P < 0.0001$ expression of sialomucins and CD10 versus sulfomucins at 6 months.
[c]$P < 0.0001$ expression of sialomucins and CD10 versus sulfomucins at 12 months.
[d]Endoscopic recurrence (Rutgeerts' score ≥1).

($P < 0.0001$) (Table 2). The expression of CD10 was higher than the expression of sulfomucins ($P < 0.0001$) and comparable with the expression of sialomucins ($P = 0.23$) (Table 2). At 6 and 12 months, findings from HC and IHC analysis did not differ when considering the whole group of 22 patients (Table 2) or the subgroup of 19 patients with available surgical samples. In this subgroup of 19 patients, the mean expression of sialomucins, sulfomucins, and CD10 at 6 months was 96.1% (60–100), 3.89% (0–40), and 94.7% (50–100), respectively. At T1, the expression of sialomucins and CD10 was comparable ($P = 0.23$) and higher than the expression of sulfomucins ($P < 0.0001$ both). At T2, the expression of sialomucins, sulfomucins, and CD10 in these 19 patients was 93.3% (60–100), 6.6% (0–40), and 88.1% (25–100), respectively. At T2, also the expression of sialomucins and CD10 was comparable ($P = 0.25$), being higher than the expression of sulfomucins ($P < 0.001$ for both).

Expression of Sialomucins, Sulfomucins, and CD10 in Patients with Versus Without Recurrence

In the whole group of 22 patients, the expression of sialomucins and sulfomucins at 6 and 12 months did not differ between patients with versus without recurrence (Table 2). The

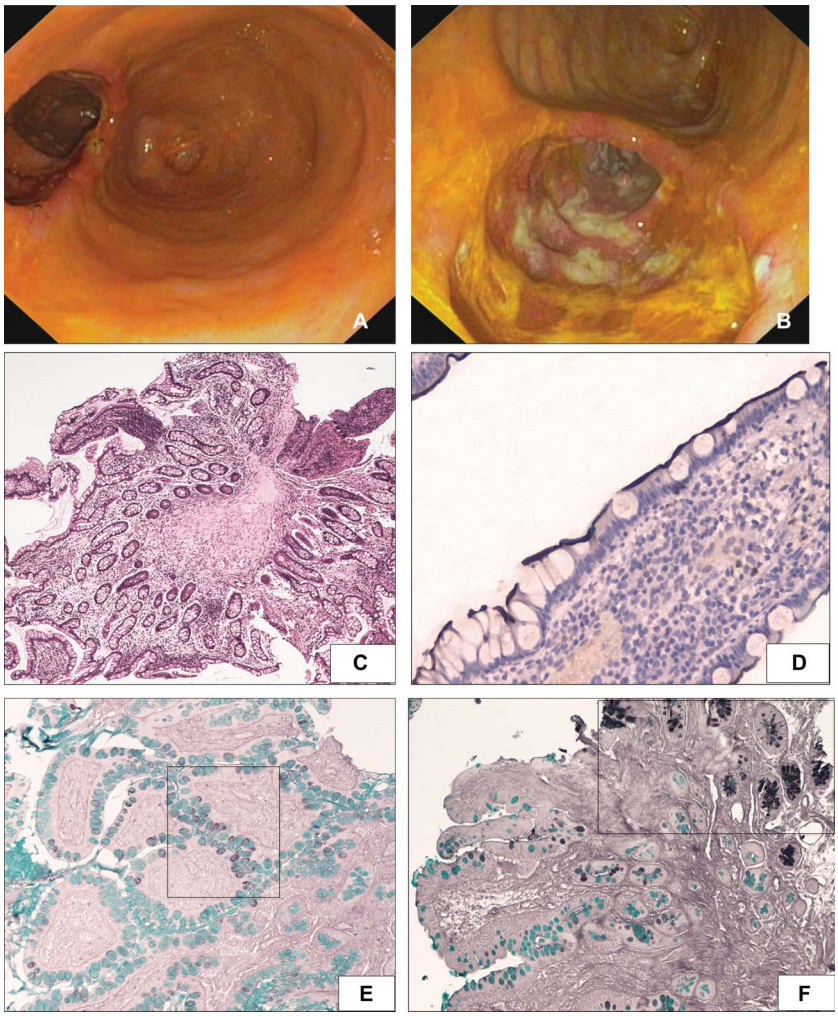

FIGURE 1. A–F, Endoscopic, histologic, and histochemical analysis from ileal samples taken at 6 (T1) and 12 months (T2) after ileocolonic resection for CD. A, At T1, ileocolonoscopy showed few apthoid ulcers (score 2). B, At T2, deep ulcerations were observed (endoscopy score 4). C, Histology sample of the neoterminal ileum taken during ileocolonoscopy at 6 months: Villi architecture is irregular (i.e., nonuniform villi); the lamina propria shows moderate inflammatory infiltrate (H&E; original magnification, ×10). D, In the same patient, the CD10 immunostain shows continuous apical immunostain, as expected in native enterocytes (CD10 immunostain), Rabbit Monoclonal Antibody at 1:50 (Diagnostic BioSystems; original magnification, ×40). E and F, Mucins' histochemical expression in the neoterminal ileum of biopsy samples taken 6 months (E; HID, original magnification, ×20) and 12 months (F; HID, original magnification, ×20) after surgery. The biopsy taken at 6 months (E) shows a predominant sulfomucins secretion (light blue stain of goblet cells). Some of the goblet cells (square) show a light brown color, as expected when a mixture of sialomucins and sulfomucins coexists. The biopsy taken at 12 months (F) shows a significant increase of the sulfomucin secretion (colonic type mucins' metaplasia, intense brown color; square).

lower percentage of expression of CD10 in patients with no recurrence at T1 was not statistically significant ($P = 0.07$). No differences were also observed between patients with versus without recurrence when considering the subgroup of 19 patients with available surgical samples (T1: sialomucins 94.9 [60–100] versus 99.4 [98–100], sulfomucins 5.07 [0–40] versus 0.6 [0–2], and CD10 100 [0–100] versus 90 [50–100]; T2: sialomucins 93 [60–100] versus 94.7 [80–100], sulfomucins 7.0 [0–40] versus 5.2 [0–20], and CD10 85 [25–100] versus 100 [100–100]; all P = not significant).

Comparison Between HC and IHC Findings at Surgery Versus 6 and 12 Months

The expression of sialomucins, sulfomucins, and CD10 was finally compared with different observations (T0 versus T1 versus T2). For a proper comparison, findings from the 19 patients with ileal samples taken not only at 6 and 12 months, but also at surgery were considered. The expression of sialomucins and CD10 (small intestinal mucin and phenotype) significantly increased in samples taken at 6 months versus surgery ($P = 0.025$ and $P = 0.026$, respectively). Differently, the mean expression of colonic mucin type (sulfomucins) significantly reduced in the ileal samples taken from the same 19 patients at 6 months versus surgery ($P = 0.027$). No other significant differences were observed. The observed trend for a higher percentage of expression of sulfomucins at T1 versus T2 was not significant ($P = 0.06$).

DISCUSSION

A genetically determined inappropriate immune response towards luminal antigens, including the resident bacterial flora, has been involved in the pathogenesis of CD.[12,13,33–36] Recurrence after ileocolonic anastomosis is more frequent than after segmental colonic resection.[37] Several trigger factors, including bile salts and the reflux of the colonic luminal content, have been suggested in the development of local inflammatory changes leading to chronicity in CD ileum.[12,13,33,35] Among these, bile salts and the reflux of the colonic luminal content.[38] A role for the luminal bacterial flora has been also reported in the pathogenesis of pouchitis.[15–22,39] Colonic metaplasia of the ileal epithelium has been described in pouchitis,[23–25,39] and changes of the luminal content in the pouch have been suggested in these findings.[24,39] The expression of colonic antigens in the neoterminal ileum has also been described after ileorectal anastomosis for CD.[28] These findings suggest that colonic metaplasia of the ileum may develop after colonic resection for both UC and CD. However, whether changes of the ileal epithelium may occur after ileocolonic resection for CD is unknown. To address this issue, the expression of small intestine and colon phenotype markers was evaluated in CD ileum before and after ileocolonic resection.

In our cohort, the frequency of clinical and endoscopic recurrence was comparable with the general CD population,[1,5,6] thus supporting the reliability of our findings. All patients referred to the same gastrointestinal and surgical units, and all the procedures were performed by the same experienced and IBD-dedicated investigators. This study design should provide a reliable assessment of the findings and improve the interobserver agreement. In all patients, ileal samples taken from both surgical and endoscopic specimens expressed sialomucins and CD10, thus suggesting a normal ileal phenotype. Accordingly, the expression of small intestine mucins and phenotype in CD ileum was higher than the expression of colonic mucins at all observations. The degree of expression of these phenotype markers, however, showed not only interindividual, but also intraindividual variations during the study period. Indeed, although the expression of both small intestine mucin and phenotype were higher in ileal samples taken at 6 months versus surgery, the expression of colonic mucin type decreased at 6 months versus surgery.

To provide a proper comparison between the expression of tested markers at different observations (surgery at 6 and 12 mo), in a subanalysis, only the 19 patients with available surgical samples were considered. Results consistently demonstrated that the ileum with severe/fully established CD-lesions (i.e., at surgery) shows a "metaplastic" colonic mucosa phenotype (i.e., sulfomicin-secreting enterocytes and loss of CD10 expression). Differently, in the same patients, the ileum showing either no recurrence or early postoperative lesions maintained both the "native" sialomucins secretion and CD10 expression, as observed in the normal ileum. To our knowledge, no comparison can be made with previous findings because data regarding the development of colonic metaplasia in CD ileum before and after ileocolonic resection are lacking. Whether metaplastic changes of CD ileum represent the result of tissue damage may be hypothesized but not demonstrated by our study aimed to first describe the possible development of colonic metaplasia in CD ileum. Nevertheless, colonic metaplasia was mainly observed in the established/severe ileal lesions, showing an increased expression of colonic mucins and a reduced expression of small intestine mucins type and phenotype. Ileal samples with no lesions or new lesions related to early recurrence indeed consistently retained the "native" ileal mucins' phenotype (sialomucins). Differently, ileal samples with severe/established lesions (i.e., at surgery) showed a reduced expression of small intestine mucin type and phenotype, associated with an increased expression of colonic mucin type. Among limitations of the study, there is the observation that although positive CD10 immunostaining is fairly specific for small intestinal enterocytes, a negative staining does not definitely exclude small intestinal mucosa. Nevertheless, the comparable findings observed when using not only CD10, but also sialomucins (as an additional marker of small intestinal phenotype) strengthen the reliability of our findings. Additional limitation of the study includes the relative short follow-up after surgery. However, the study protocol included clinical and endoscopic assessment at 6 and 12 months, according to most of the studies assessing the natural history of recurrence after ileocolonic resection for CD.[1–6,9–11] The small number of patients not showing endoscopic recurrence at 6 and 12 months did not allow to address whether the development of colonic metaplasia may be related or may predict CD recurrence. This observation may also account for the comparable findings observed in patients with or without

endoscopic recurrence. In preliminary experiments, additional marker(s) were searched (antihuman tropomyosin 5),[24] but our research then focused on tested markers to provide a more clear initial message regarding colonic metaplasia and CD.

These findings demonstrate that colonic metaplasia may develop in the ileum with severe/established lesions related to CD. This is consistently supported not only by the loss of the native small intestine phenotype (sialomucins and CD10), but also by the increased colonic type mucin (sulfomucins) secretion in the involved ileum. Supporting this concept, the neoterminal ileum showing no recurrence or early postoperative recurrence normally expressed the small intestine type-mucins and phenotype. Larger studies are required to further address this issue. The study protocol indeed required several clinical and endoscopic assessments early after surgery. The feasibility of the study and development of colonic metaplasia in CD ileum was therefore first assessed in a subgroup of patients. On the basis of these encouraging findings, ongoing larger studies using additional markers are currently investigating the possible relationship between colonic metaplasia and tissue damage in CD ileum.

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