

Mycophenolate Mofetil-related Gastrointestinal Mucosal Injury: Variable Injury Patterns, Including Graft-versus-Host Disease-like Changes

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Abstract: Mycophenolate mofetil (MMF) is a commonly used immunosuppressive drug used in the management of transplant recipients. Although gastrointestinal (GI) toxicity is a known complication of MMF, the literature characterizing the pathologic features of MMF in the GI tract is sparse. This study characterizes the pathologic features of MMF toxicity in both the upper and lower GI tract, correlating it with clinical and endoscopic findings. Seventy-five GI biopsies (9 esophageal, 15 gastric, 16 duodenal, 5 ileal, 30 colonic) from 46 transplant recipients from 2002 to 2006 were obtained and assessed for multiple histologic features. Clinical features were recorded for all cases and endoscopic findings. Only MMF patients showed ulcerative esophagitis (5/7 cases) and reactive gastropathy (4/10 cases). Only MMF patients showed graft-versus-host disease (GVHD)-like features in duodenal (4/12 cases) and ileal (1/5 cases) biopsies. GVHD-like changes were seen more frequently among patients on MMF compared with those not on MMF [9 (56%) vs. 2 (14%); $P = 0.017$]. Crypt architectural disarray [12 (75%) vs. 2 (14%); $P = 0.001$], lamina propria edema [9 (56%) vs. 2 (14%); $P = 0.017$], increased lamina propria inflammation [13 (81%) vs. 3 (21%); $P = 0.001$], dilated damaged crypts [7 (44%) vs. 1 (7%); $P = 0.024$], and increased crypt epithelial apoptosis [9 (56%) vs. 2 (14%); $P = 0.017$] were more common with MMF patients compared with non-MMF patients. In conclusion, pathologists should be aware of the potential manifestations of MMF toxicity throughout the GI tract, including ulcerative esophagitis, reactive gastropathy, and GVHD-like features in intestinal biopsies.

Key Words: drugs, gastrointestinal, MMF, mycophenolate mofetil, pathology, toxicity

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Mycophenolate mofetil (MMF) has become an important and commonly used drug in the maintenance of immunosuppressive therapy for recipients of all types of organ transplants. MMF is an antimetabolite

drug, which blocks the de novo pathway of purine synthesis.² Although it selectively inhibits B and T lymphocyte proliferation, enterocytes are partially susceptible to MMF and one of the main limitations of this drug is gastrointestinal (GI) toxicity.² Diarrhea is the most frequently reported adverse effect, which may be a cause of patient noncompliance or physician-directed drug cessation, and also a wasting-type illness in severe cases.^{6,9}

Preliminary studies have described histologic abnormalities in the colon associated with MMF, with graft-versus-host disease (GVHD)-like, inflammatory bowel disease (IBD), or Crohn-like changes, as well as erosive or ulcerative enterocolitis.^{3,7,8,13–16} The literature describing upper GI tract pathology in MMF patients is sparse, despite the relative frequency of upper GI symptoms in these patients.

In the present study, we characterize the pathologic features of MMF toxicity in both the upper and lower GI tract, correlating it with patient symptomatology and endoscopic findings. Pathologists should be aware of the potential manifestations of MMF toxicity throughout the GI tract, so as to avoid incorrect diagnoses and provide information that may lead to alteration of a patient's immunosuppressive regimen.

MATERIALS AND METHODS

The files of the Pathology Department of London Health Sciences Centre were searched for all GI biopsies from transplant recipients from June 2002 to June 2006. Patients who had received either a bone marrow transplant or a bowel transplant were excluded, because features of GVHD or bowel rejection overlap with features hypothesized to occur with MMF. Patients with known IBD were excluded for similar reasons.

A total of 46 patients were accrued. There were 16 patients who underwent upper endoscopy only, 26 patients who underwent lower endoscopy only, and 4 patients who underwent both upper and lower endoscopy. Of the 20 patients who underwent upper endoscopy, there were 12 kidney transplant recipients, 6 liver transplant recipients, 1 combined heart/kidney transplant recipient, and 1 combined pancreas/kidney transplant recipient. Of the 30 patients who underwent lower endoscopy, there were 13 kidney transplant recipients, 10 liver transplant recipients, 3 heart transplant recipients, 1 lung transplant recipient,

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2 combined heart/kidney transplant recipients, 1 combined pancreas/kidney transplant recipient.

Multiple histologic features were assessed in each of the biopsies by Jeremy R. Parfitt and David K. Driman, with specific features depending upon the site of the biopsy. Within esophageal and gastric biopsies, architectural and reactive changes were assessed, including squamous hyperplasia in the esophagus and foveolar hyperplasia and smooth muscle proliferation in the stomach. A diagnosis of reactive gastropathy was made when a constellation of features, including foveolar hyperplasia, foveolar mucin depletion, lamina propria edema, smooth muscle proliferation, and congestion were present. Additionally, erosions or ulcers, increased or decreased inflammation, epithelial apoptosis, and infectious organisms were assessed for in all esophageal and gastric biopsies.

Within large and small intestinal biopsies, a biopsy was considered to show GVHD-like features when it showed a constellation of features, including mild crypt architectural disarray or villous blunting, edema, and increased crypt epithelial apoptosis. Crypt epithelial apoptosis was considered to be increased when the apoptotic rate was greater than 5/100 crypts.¹² A colonic biopsy was considered to be IBD-like when it showed marked crypt architectural disarray and markedly increased lymphocytes and plasma cells in the lamina propria. Lamina propria lymphocytes and plasma cells were considered to be increased when they were present in sheets, with loss of the normal gradient of decreasing chronic inflammation from the surface to the base of the lamina propria. An additional feature in intestinal biopsies with MMF effects that was observed during preliminary assessment was the presence of dilated damaged crypts (DDCs), which differ from typical crypt abscesses seen in IBD in that they consist of cystically dilated crypts with a flattened epithelial lining and few intraluminal neutrophils or apoptotic debris. These features, as well as erosions or ulcers, cryptitis, crypt loss, and infectious organisms were assessed for in all intestinal biopsies. Statistical analysis consisted of χ^2 tests and statistical significance was determined with an α level P value of < 0.05 .

Clinical features were recorded for all cases, including patients' demographic details, organ of transplantation, medications, past medical history, reason for endoscopy, and endoscopic findings. In particular, attention was given to whether or not the patient was on MMF at the time of endoscopic biopsy and the dose of the drug.

RESULTS

Clinical Features

Of 20 patients who underwent upper endoscopy, 14 were on MMF. The mean patient age was 53 years; there were 8 males and 12 females. Dyspepsia, often with nausea and vomiting, was the most common presentation and occurred with similar frequency, regardless of whether or not the patients were on MMF (8/14 patients on MMF; 6/6 patients not on MMF). There were 3

patients with swallowing difficulties, including odynophagia and dysphagia, all of whom were on MMF. Additionally, 2 patients presented with symptoms of upper GI bleeding, such as melena, both of whom were on MMF. Endoscopically, no abnormalities were seen in 7 of 14 patients on MMF and 3 of 6 patients not on MMF, whereas nonspecific gastritis or duodenitis were seen with similar frequency among patients, regardless of whether or not they were on MMF (4/14 patients on MMF; 2/6 patients not on MMF). Esophagitis with ulceration was seen in the 3 patients on MMF with swallowing problems and the 2 patients on MMF with melena were found to have duodenal ulcers.

Of 30 patients who underwent colonoscopy, 16 were on MMF. The mean patient age was 53 years; there were 19 males and 11 females. Diarrhea, which was the most common indication for colonoscopy, was a more frequent complaint among patients on MMF compared with those not on MMF [12 (75%) vs. 5 (35%); $P = 0.03$]. Diarrhea was also more frequent among patients on FK506 compared with those not on FK506 [14 (74%) vs. 4 (33%); $P = 0.03$]. Whether on MMF or FK506, the diarrhea was usually described as being watery in nature. There was no statistically significant relationship between other immunosuppressive drugs and diarrhea. The remaining patients underwent colonoscopy for either nonspecific abdominal discomfort, rectal bleeding, or as part of a routine surveillance program. Endoscopically, no abnormalities were seen in 10 of 16 patients on MMF, whereas nonspecific, patchy erythema was seen in the remaining patients. Endoscopically, no abnormalities were seen in 11 of 14 patients not on MMF, whereas a strangulated hernia was seen in 1 patient and focal, nonspecific ulceration and erythema were seen in 2 others.

Pathologic Features

Esophagus

There were 7 esophageal biopsies from patients on MMF and 2 from patients not on MMF. Of the 7 biopsies from patients on MMF, 5 showed variable degrees of active esophagitis, with erosive or ulcerative esophagitis in 4 cases. In one of these biopsies, there were features of herpes esophagitis, whereas in the remaining 4 cases, no etiology was apparent with hematoxylin and eosin and periodic acid-Schiff (PAS) stains. The 2 biopsies from patients not on MMF were normal.

Stomach

There were 10 gastric biopsies from patients on MMF and 5 from patients not on MMF. Of the 10 biopsies from patients on MMF, 4 showed moderate-to-severe reactive gastropathy and 1 showed Crohn-like features, including granulomatous inflammation (Fig. 1); no organisms were identified with Ziehl Neelsen and PAS stains. In the remaining 5 cases, there were no significant abnormalities. There was no history of aminosalicylic acid or nonsteroidal anti-inflammatory drug (NSAID) use among patients whose biopsies

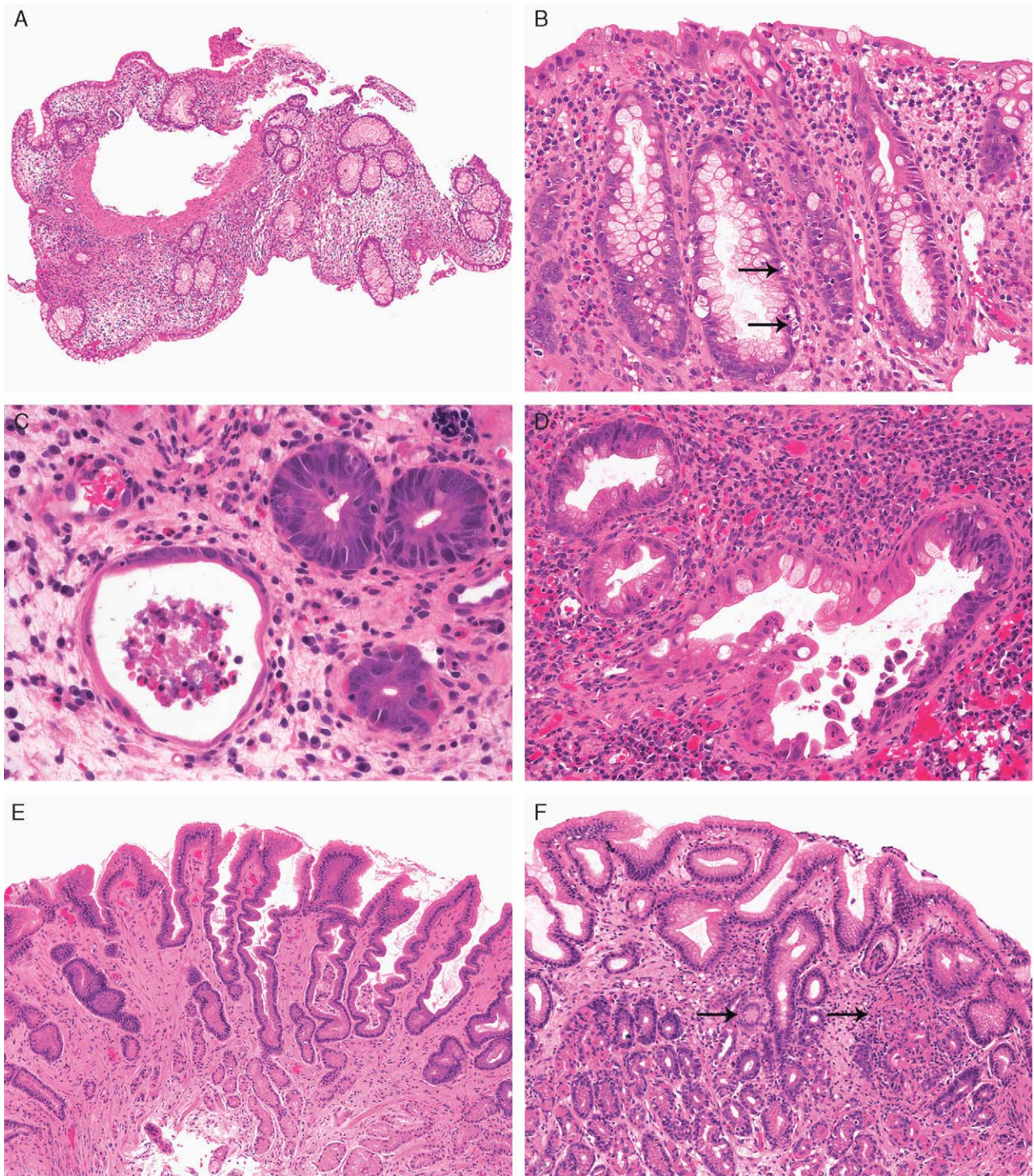


FIGURE 1. Features of mycophenolate mofetil-induced injury: crypt architectural disarray and loss (A), increased crypt apoptotic bodies (B, arrows), dilated damaged crypt with cystic dilation, flattened epithelial lining, intraluminal neutrophils, and apoptotic debris (C), irregular crypts with partial disruption of the lining epithelium and cryptitis (D), reactive gastropathy changes (E), gastric granulomatous inflammation (F, arrows).

showed features of reactive gastropathy and these patients were not on any other drugs known to cause reactive gastropathy. The 5 biopsies from patients not on

MMF showed an incidental hyperplastic and fundic gland polyp in 2 cases, but no significant abnormalities in the remaining cases.

Duodenum

There were 12 duodenal biopsies from patients on MMF and 4 from patients not on MMF. Of the 12 biopsies from patients on MMF, 4 showed GVHD-like changes, including a variable degree of villous blunting, lamina propria edema, DDCs, increased crypt epithelial apoptosis, and mild, patchy neutrophilic inflammation. In 1 case, there were Crohn-like features, including granulomatous inflammation. In 1 case, there were features of florid *Mycobacterium avium intracellulare*-associated duodenitis, evident with Ziehl Neelsen and PAS stains. The remaining 6 cases were either normal or, in 1 case, showed slightly increased lamina propria lymphocytes and plasma cells. The 4 biopsies from patients not on MMF were either normal or, in 1 case, showed slightly increased lamina propria lymphocytes and plasma cells.

Ileum

There were 5 biopsies from the terminal ileum, all from patients on MMF. Four of the 5 biopsies showed abnormalities. Two cases showed GVHD-like features, whereas another case showed Crohn-like features, including granulomatous inflammation. No organisms were identified with Ziehl Neelsen and PAS stains. The other abnormal biopsy showed active ileitis and one of the ileal biopsies was normal.

Colon

The pathologic features of MMF effects in colonic biopsies are summarized in Table 1 and illustrated in Figure 1. GVHD-like changes were seen more frequently among patients on MMF compared with those not on MMF [9 (56%) vs. 2 (14%); $P = 0.017$]. Of the 9 GVHD-like cases, colonoscopic examination was normal in 7 cases and patchy erythema was reported in 2 cases. Crypt architectural disarray [12 (75%) vs. 2 (14%); $P = 0.001$], lamina propria edema [9 (56%) vs. 2 (14%); $P = 0.017$], increased lamina propria inflammation [13 (81%) vs.

3 (21%); $P = 0.001$], DDCs [7 (44%) vs. 1 (7%); $P = 0.024$], and increased crypt epithelial apoptosis [9 (56%) vs. 2 (14%); $P = 0.017$] were more common with MMF patients compared with non-MMF patients. The crypt architectural disarray in biopsies with MMF effects was milder than in typical cases of IBD. The edema was typically more prominent than the inflammation, which usually consisted of patchy foci of neutrophils, giving an empty appearance to the lamina propria. The presence of DDCs was a relatively specific finding seen in biopsies with MMF effects and the flattened epithelial lining sometimes showed prominent regenerative atypia. There were only 2 cases with features that were similar to IBD, which, in addition to architectural changes, showed markedly increased lamina propria inflammation and, in 1 case, granulomatous inflammation; no organisms were identified with Ziehl Neelsen and PAS stains. The frequency of erosions or ulcers, cryptitis, and crypt loss was similar among patients, regardless of whether or not they were on MMF. The frequency of usage of other immunosuppressive drugs, including FK506, was similar among both the MMF and non-MMF patient groups (data not shown) and patients whose biopsies showed GVHD-like or IBD-like features were not on any other drugs known to cause these changes. Only 1 biopsy was from a patient on enteric-coated mycophenolate, which appeared normal. There was no difference in MMF dosage between patients on MMF with GVHD-like or IBD-like features and those on MMF without these features (data not shown).

DISCUSSION

This study shows that MMF, a commonly used immunosuppressive drug known to cause dyspepsia and diarrhea, shows pathologic features throughout the GI tract. In the upper GI tract, effects reflect local mucosal irritation with ulcerative esophagitis, reactive gastropathy type changes, and duodenal ulcers. In the lower GI tract, effects reflect antimetabolite activity, giving rise to GVHD-like changes of MMF colitis.

Within the upper GI tract, MMF toxicity seems to be similar to that of NSAIDs, an important cause of upper GI erosions and ulcers, and also reactive gastropathy. Ulcerative esophagitis (3/7 MMF patients), reactive gastropathy (4/10 MMF patients), and upper GI bleeding because of duodenal ulcers (2/12 MMF patients) were present only in patients on MMF. With the exception of 1 patient with herpes esophagitis, no morphologic or clinical cause for these cases of ulcerative esophagitis, reactive gastropathy, and duodenal ulcers was apparent, and none of these patients were on NSAIDs, aminosalicylic acid, or any other drugs known to cause these changes.

MMF and NSAIDs have similar physicochemical properties and both seem to induce topical damage to the GI tract.¹ Studies have shown that the incidence of dyspepsia with either MMF or NSAIDs is approximately 40% to 45%, whereas the incidence of ulceration and

TABLE 1. Pathologic Features of MMF in Colonic Biopsies

Morphologic Feature	MMF Patients (n = 16)	Non-MMF Patients (n = 14)	P
	n (%)	n (%)	
Crypt architectural disarray	12 (75)	2 (14)	0.001
Erosions/ulcers	4 (25)	2 (14)	> 0.05
Lamina propria edema	9 (56)	2 (14)	0.017
Increased lamina propria inflammation	13 (81)	3 (21)	0.001
Cryptitis	6 (37)	3 (21)	> 0.05
Dilated damaged crypts	7 (44)	1 (7)	0.024
Crypt loss	4 (25)	2 (14)	> 0.05
Increased crypt epithelial apoptosis	9 (56)	2 (14)	0.017
GVHD-like changes	9 (56)	2 (14)	0.017
IBD-like changes	2 (13)	0 (0)	> 0.05

GVHD indicates graft-versus-host disease; IBD, inflammatory bowel disease; MMF, mycophenolate mofetil

bleeding is greater for MMF (3% to 8%) compared with NSAIDs (0.5% to 4%).¹ We are not aware of any studies demonstrating MMF as a cause for reactive gastropathy.

Noninfectious esophageal ulcers in transplant recipients are uncommon. Farrell et al⁵ described idiopathic giant esophageal ulcers in a renal transplant patient, which responded to steroids. Duodenal pathology attributed to MMF is uncommon. Ducloux et al⁴ described villous atrophy with crypt hyperplasia in a patient on MMF with severe diarrhea, who had no serologic evidence of celiac disease, and suggested that the duodenal pathology was the cause of the diarrhea.

Given the apparent topical toxicity of MMF within the upper GI tract, enteric-coated forms of the drug have been developed to reduce irritative effects on GI mucosa. Preliminary studies comparing MMF with enteric-coated mycophenolate sodium have not shown a significant reduction in either upper or lower GI tract adverse outcomes.¹ In our study, there was only 1 patient on enteric-coated mycophenolate; the patient underwent colonoscopy only and no endoscopic or pathologic abnormalities were found.

Watery diarrhea, which was present in approximately half of all patients, was the most common complaint among patients who underwent lower endoscopy and patients who were on MMF reported this symptom more frequently than those not on MMF. Patients who were on FK506 also complained of diarrhea more frequently than those not on FK506. Other studies of GI complications in solid organ transplant recipients have also reported that diarrhea occurs in approximately half of the patients, especially in those on either MMF or FK506.^{6,9} However, only biopsies from MMF patients showed pathologic abnormalities in our study. Most colonoscopies were normal, even among MMF patients, with patchy erythema being present only in a minority of patients. As such, MMF colitis is conceptually similar to other forms of "microscopic colitis," as 7 of 9 colonoscopies from MMF patients with histologic GVHD-like features were normal.

Histologically, MMF colitis is characterized by GVHD-like features, including mild crypt architectural disarray, prominent lamina propria edema, giving biopsies an "empty" appearance, DDCs, and increased crypt epithelial apoptosis. Patchy foci of neutrophils may also be seen in some cases. Although most of these features are relatively nonspecific on their own, DDCs are a feature of antimetabolite drug-induced colitis. DDCs are characterized by cystically dilated crypts with flat or atrophic epithelial lining, sometimes showing regenerative or bizarre atypia, and lumens that are either empty or contain sparse neutrophils or debris. The DDCs, together with increased crypt epithelial apoptosis, suggest an acute or subacute phase of antimetabolite drug-induced epithelial injury, whereas the crypt architectural disarray, with occasional crypt loss, suggests chronicity or previous episodes of injury with partial healing.

MMF is subject to near complete metabolic conversion to its active component, mycophenolic acid (MPA), within the liver.² MPA is a potent inhibitor of

inosine monophosphate dehydrogenase, a key enzyme in the de novo pathway of purine synthesis.² Lymphocyte proliferation, which is 90% dependent on this pathway of purine synthesis, is selectively inhibited by MPA, which makes MMF an effective immunosuppressant.² However, enterocytes are 50% dependent on the de novo pathway of purine synthesis, which accounts for their selective vulnerability to MMF's antimetabolite effects within the GI tract.² Studies have shown that MMF may reduce the crypt proliferative index, which may be detected as reduced Ki-67 immunostaining in biopsies and clinically may result in impaired healing of colonic anastomoses.^{3,17}

Dalle et al³ studied the pathologic features in 24 colonic biopsies of patients on MMF. They reported discontinuous crypt architectural changes, increased epithelial mucin secretion, mild active inflammation, focal dilated and inflamed crypts, but no increase in apoptosis. These changes were interpreted as being Crohn-like. In our study, IBD-like changes were seen in only 2 biopsies from MMF patients. When Papadimitriou et al¹⁴ studied the histologic features of MMF-related colitis, they found that apoptosis, enterocyte atypia, increased neuroendocrine cells, and architectural distortion were more prominent among 20 MMF patients compared with 8 controls. They also compared the findings in MMF patients with GVHD patients and IBD patients and concluded that MMF-related colitis was more similar to GVHD than IBD, but apoptosis was milder than in GVHD patients. Selbst et al¹⁶ described features of MMF effects in 18 colonic biopsies. Architectural distortion and apoptosis were associated with MMF. There were 2 cases with severe apoptosis considered to be GVHD-like, whereas 2 cases showed architectural distortion and inflammation considered to be IBD-like. Other isolated cases of pseudomembranous colitis and ischemic colitis attributed to MMF have also been reported in the literature.^{10,11}

In summary, MMF has the potential to cause toxic injury throughout the GI tract. Within the upper GI tract, MMF appears to cause topical irritation and damage, leading to NSAID-like pathology, including esophageal and duodenal ulcers, and also reactive gastropathy, which appears to be a common manifestation of MMF. Within the lower GI tract, MMF-induced pathology appears to be related mainly to its antimetabolite effects, giving biopsies from the colon and ileum GVHD-like properties, including mild architectural changes with DDCs, lamina propria edema, increased crypt epithelial apoptosis, and patchy neutrophilic inflammation. In the posttransplant setting, DDCs in a colorectal biopsy should raise a pathologist's index of suspicion for MMF effects. Pathologists should be aware of MMF colitis, so as to avoid erroneous diagnoses, such as GVHD or IBD, and provide information, which may lead to alteration of immunosuppressive therapy.

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