

Graft-versus-Host Disease-Like Pattern in Mycophenolate Mofetil Related Colon Mucosal Injury: Role of FISH in Establishing the Diagnosis

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Key Words

Mycophenolate mofetil · Graft-versus-host disease · Fluorescence in situ hybridization · Heart transplantation · Colitis

Abstract

Mycophenolate mofetil (CellCept®), a commonly used immunosuppressive drug in solid organ transplantation, has recently been shown to cause graft-versus-host disease (GVHD)-like changes in the gastrointestinal tract. On rare occasions, true GVHD has also been documented in the gastrointestinal tract of solid organ transplant patients. Because the treatment for these two entities is different, i.e. removal of the offending agent versus the administration of steroids, proper identification of the cause is imperative. We present a case of mycophenolate mofetil colitis mimicking grade I GVHD of the gut. In our study, we used fluorescence in situ hybridization for the Y chromosome to document the lack of male donor lymphocytes in the female recipient colon biopsy. We suggest that molecular techniques including fluorescence in situ hybridization could be used to discriminate between MMF-related colitis and true GVHD in order to help guide therapy.

Introduction

Mycophenolate mofetil (MMF), a commonly used immunosuppressive drug in solid organ transplantation, has resulted in a significant decrease in allograft rejection. MMF is an antimetabolite that inhibits inosine monophosphate dehydrogenase, resulting in blockade of the de novo pathway of purine synthesis. Because this pathway is exclusively

used by B and T lymphocytes for purine synthesis, MMF administration causes selective inhibition of lymphocyte proliferation [1]. Gastrointestinal side effects, including diarrhea, are common with MMF and are caused by specific (suppression of de novo purine synthesis) and nonspecific (immunosuppressive) effects of the drug on the gastrointestinal tract [1]. MMF-related colitis has many features in common with graft-versus-host disease (GVHD) colitis seen in patients who have undergone allogeneic bone marrow transplantation, including crypt architectural disarray, gland distortion with lamina propria fibrosis and edema, increased lamina propria inflammation, and increased crypt epithelial apoptosis [2–4]. Some have also found enterocyte atypia, increased neuroendocrine cells, and microvascular injury [2, 3]. We report a case of colitis with features consistent with both MMF colitis and GVHD in a patient receiving MMF as part of an immunosuppressive regimen after heart transplantation. Because the recipient was female and the donor was male, we used fluorescence in situ hybridization (FISH) for the Y chromosome to determine the origin of the lymphocytes present within the colon biopsy. The presence or absence of donor lymphocytes in colonic lesions in conjunction with clinical data could help to determine the cause of the patient's colitis and guide treatment.

Case Report

The patient was a 69-year-old woman with a past medical history significant for osteopenia, chronic lower back pain and diverticulosis who presented with weakness and fatigue for approximately one month accompanied by low blood pressure (70/40 mm Hg) and mild shortness of breath. The patient had a history of a left heart catheterization in 2007 which showed dilated nonischemic cardiomyopathy with an ejection fraction of approximately 30%. On the last admission prior to transplantation, the patient was hypotensive and had an ejection fraction of 15%. She was started on dobutamine, and an implantable cardioverter-defibrillator device was placed. The patient was ultimately maintained on milrinone and evaluated for orthotopic heart transplantation.

Once a heart became available for transplantation, the patient was started on prednisone, MMF, and tacrolimus. A heart from a male donor was transplanted without complication. The explanted heart showed dilated cardiomyopathy with cardiomyopathy of end-stage heart failure. The patient's postoperative course was complicated by an upper extremity deep venous thrombosis for which the patient received enoxaparin 60 mg subcutaneous every 12 h. The patient was discharged to home on postoperative day eight on valgancyclovir, pravastatin, iron sulfate, enoxaparin, nystatin, diltiazem-SR, Bactrim-DS, aspirin, lexapro, and an immunosuppressive regimen including tacrolimus 4 mg every 12 h, a prednisone taper, and MMF 750 mg every 12 h.

Two months after transplantation, the patient presented with a two-week history of watery stools and diarrhea consisting of four to five episodes after each meal with occasional maroon blood in the stool. These episodes were preceded by cramping, nonradiating abdominal pain and were not accompanied by fevers, chills, nausea, vomiting, hematemesis, melena, chest pain, shortness of breath, dizziness, palpitations, dysuria, or hematuria. She had a history of hemorrhoids and diverticulitis in the past and had also had a recent colonoscopy which was negative. There was no history of NSAID use or peptic ulcer disease. Microscopic studies of her stool were negative for *Giardia lamblia*, *Entamoeba histolytica*, ova and parasites, *Salmonella* spp., *Shigella* spp., *Cryptosporidium*, *Campylobacter* spp., as well as *C. difficile* toxin and antigen and *Rotavirus* antigen. There was no increase in white blood cells in the stool. The stool was Hemocult-positive. An abdominal X-ray demonstrated no intestinal obstruction. A colonoscopy performed at this time showed severe diverticulosis, and biopsies revealed colonic mucosa with crypt apoptosis, lamina propria fibrosis, and inflammatory cell infiltrate which could be consistent with GVHD. No evidence of cytomegalovirus was identified. The patient was treated with three days of outpatient steroids. Her MMF was held prior to discharge due to leukopenia.

The patient was readmitted three days later with bloody, Hemocult-positive stools. Colonoscopy and esophagogastroduodenoscopy failed to demonstrate a source of bleeding. It was felt that the patient's symptoms, including bleeding and bloating, were due to MMF, and her immunosuppressive regimen was changed to tacrolimus and azathioprine with amelioration of her gastrointestinal symptoms.

FISH for the X and Y chromosome was performed on paraffin-embedded tissue prepared in the histology lab at Thomas Jefferson University Hospital. The slides were subjected to deparaffinization using Hemo-D (three washes, 10 min each) and 100% ethanol (two washes, 5 min each). The slides were pretreated as follows: 0.2 N HCl for 20 min at room temperature followed by a wash in 2× SSC for 3 min at room temperature; 2× SSC at 73°C for 30 min; 30% pepsin in 2× SSC for 30 min at 45°C followed by a wash in 2× SSC for 3 min at room temperature; proteinase K for 20 min at 45°C followed by a wash in 2× SSC for 3 min at room temperature. The slides were postfixed in 10% formalin for 10 min at room temperature and washed in 2× SSC for 3 min at room temperature. The slides were dehydrated and denatured and prepared for hybridization with CEP X and CEP Y Dual color probe (1:10 dilution) (Vysis, Des Plaines, Ill., USA) overnight at 37°C. The slides were washed in posthybridization buffer for 2 min at 72°C and counterstained with 4',6-diamidino-2-phenylindole.

Microscopic examination of the random colon biopsies revealed colonic mucosa with areas of crypt apoptosis and lamina propria fibrosis (fig. 1). There was a mixed inflammatory cell infiltrate predominantly composed of mature lymphocytes. No viral inclusions were identified, and a special immunohistochemical stain for cytomegalovirus was negative (data not shown).

Because the patient was female and the donor was male, FISH was performed to detect the presence of the Y chromosome in lymphocytes present in the biopsy material. Gender-mismatched transplantations, in which a female patient receives a male donor organ, offer a unique opportunity to investigate recipient tissues, because the detection of the Y chromosome discriminates the cells of donor origin from recipient-derived cells. No Y chromosome signals were detected, indicating there was no significant infiltrate by cells derived from the male heart donor (fig. 1).

Discussion

MMF, a commonly used immunosuppressive drug for solid organ transplantation, has resulted in a dramatic decrease in graft rejection. MMF acts by inhibiting inosine monophosphate dehydrogenase resulting in selective inhibition of the de novo pathway for purine synthesis. Purine synthesis can occur via the de novo and/or the salvage pathway in most cells. However, lymphocytes are unique in that they almost exclusively use the de novo pathway. Therefore, administration of MMF results in selective inhibition of lymphocyte proliferation [1].

Gastrointestinal side effects are common with MMF administration. These effects are due to specific and nonspecific effects of MMF on the gastrointestinal tract. One nonspecific effect is increased immunosuppression leading to increased susceptibility of the gastrointestinal mucosa to infection by microorganisms and viruses. Increased immunosuppression does not increase susceptibility to any single organism but results in more likely infection with less pathogenic organisms as well as increased symptomatology in cases where infections may have remained subclinical [1].

MMF also has specific effects on enterocytes. These cells are approximately 50% dependent on the de novo pathway for purine synthesis. Because enterocytes have a high turnover rate, inhibition of proliferation by MMF can have a dramatic effect on mucosal integrity. Interestingly, after administration, the highest concentrations of MMF have been found in the gastrointestinal tract, which may exacerbate the effect of MMF on these cells [1].

There is some evidence that in lymphocytes, with increasing exposure to MMF there is induction of inosine monophosphate dehydrogenase expression which may counteract the effects of MMF. Some feel that this may also occur in enterocytes which may explain the decrease in additional incidence of gastrointestinal side effects after 6 months of MMF usage [1].

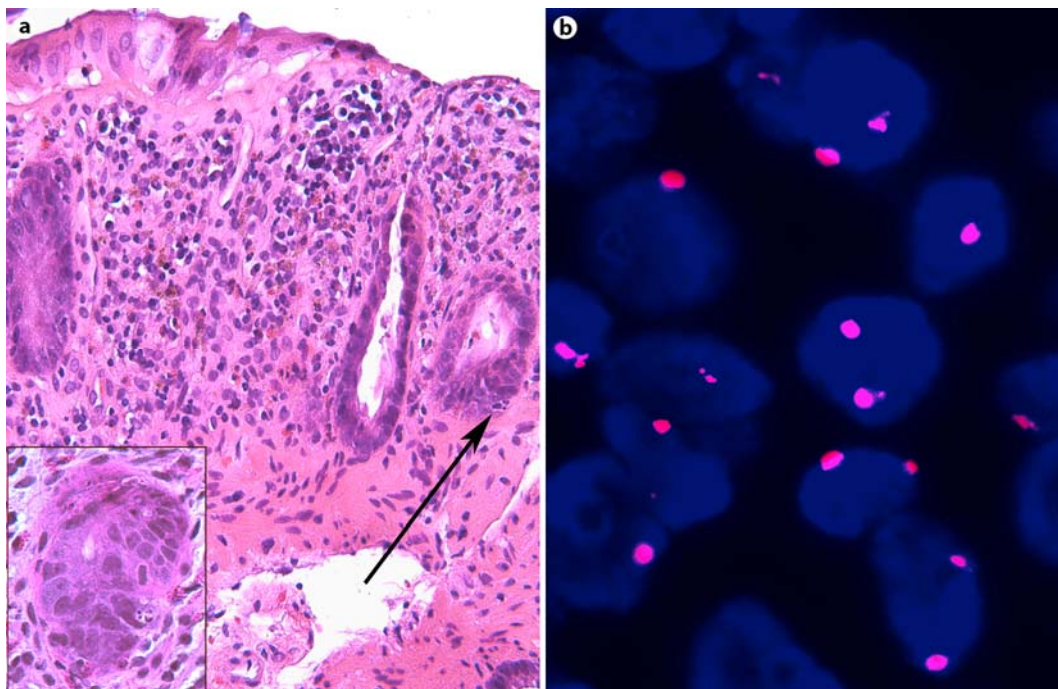
There has been limited examination of the histologic findings associated with MMF-related colitis with several groups reporting GVHD-like histology [2–4]. In one study, MMF colitis showed a significant difference in crypt cell apoptosis, gland distortion, epithelial cell atypia, the number of neuroendocrine cells, and lamina propria inflammation as compared to matched controls. This morphology significantly overlaps with that seen in GVHD [2]. A later study demonstrated that despite the overlap in histologic features, certain findings such as crypt cell apoptosis and the number of apoptotic cells were increased with GVHD as compared to MMF exposure. This study also found that mucosal architectural disarray, epithelial cell atypia, lamina propria inflammation, number of intraepithelial lymphocytes, number of neuroendocrine cells, mononuclear cell apoptosis, and microvascular injury were also increased in both patients with GVHD and those exposed to MMF as compared to controls [3]. A recent study showed that in patients exposed to MMF, GVHD-like histology can be seen throughout the gastrointestinal tract including the stomach and esophagus [5].

Some may argue that the histologic findings that some are attributing to MMF toxicity may actually be due to GVHD. As opposed to bone marrow transplantation, GVHD is uncommon in solid organ transplantation [6–8]. Billingham [9] suggested that three criteria must be met in order for GVHD to occur: (1) presence of HLA differences between the host and donor, (2) presence of immunocompetent donor cells, and (3) suppression of the host immune system. These criteria are easily met in bone marrow transplants that are not HLA-matched since a competent immune system is transplanted along with other marrow elements necessary for effective hematopoiesis. In solid organ transplantation, a small number of immunocompetent cells are present in transplanted organs. Also, due to logistical concerns, the organs are often not HLA-matched. Some solid organs carry more lymphoid tissue, which explains the larger number of reported cases of GVHD in liver, small bowel, and heart-lung as opposed to kidney and heart transplantations [6, 7]. Immunocompetent cells within the donor organ migrate into host tissues relatively quickly after transplantation. GVHD in these cases usually occurs 1–12 weeks after transplantation [6]. The presence of donor lymphocytes has been documented in recipient tissue using HLA antibodies and short tandem repeat analysis [6–8]. The presence of these lymphocytes has correlated with the presence of GVHD in these recipients [6–8].

In the current case, the patient began to experience diarrhea approximately 8 weeks after transplantation. Infectious etiologies were ruled out, and a colon biopsy demonstrated findings consistent with grade 1 GVHD. Because the recipient was female and the donor was male, we were able to determine the origin of lymphocytes present within the colon biopsy using a probe for the Y chromosome. We decided against using short tandem repeat analysis, which is commonly employed for bone marrow engraftment studies, because the colon biopsy was paraffin-embedded, which would have reduced our ability to adequately amplify DNA present within the biopsy. Examination of the peripheral blood for donor lymphocytes was not performed due to the unavailability of this specimen at the time of our study. Ultimately, we showed that there was no significant donor lymphocyte infiltrate in the recipient colon biopsy. This finding together with the improvement of the patient's symptoms subsequent to removal of MMF from the patient's immunosuppressive regimen suggest that MMF toxicity, as opposed to GVHD, was the cause of the patient's colitis. Although the patient was also administered steroids subsequent to the colon biopsy for possible GVHD, we feel that given the lack of donor lymphocytes in the colon biopsy, the rapid improvement in symptoms subsequent to MMF withdrawal, and the rarity of GVHD in heart transplantation, MMF toxicity is a much more likely cause of the patient's symptomatology.

In the current case, we found that there is substantial overlap between the histologic findings present in GVHD and MMF-related colitis. Molecular techniques, including FISH where appropriate, can be employed to demonstrate the presence or absence of donor lymphocytes. Molecular findings properly correlated with clinical and morphologic findings can help direct therapy which may include withdrawal of the offending agent, in the case of MMF-related colitis, or administration of steroids, in the case of GVHD.

Fig. 1. GVHD-like histology in MMF toxicity of the colon. **a** There is a mixed inflammatory infiltrate composed primarily of mature lymphocytes and eosinophils. The arrow indicates an apoptotic enterocyte within a crypt. The inset shows a higher magnification of a crypt with an apoptotic enterocyte (hematoxylin-eosin, original magnifications 200× (inset 400×)). **b** FISH for detection of the X (red) and Y (green) chromosome (XX/XY) in lymphocytes of the lamina propria. Only the X chromosome is detected in lymphocytes of the lamina propria in this specimen.



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