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ARTICLE

Cytomegalovirus reactivation in inflammatory bowel disease: an uncommon occurrence related to corticosteroid dependence

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Abstract

Cytomegalovirus promotes mucosal injury in patients with inflammatory bowel disease, historically affecting 10–25% of ulcerative colitis patients with refractory disease. Viral reactivation is likely related to long-term corticosteroid therapy, which is no longer central to maintenance of patients with inflammatory bowel disease. We hypothesize that viral detection rates have decreased in the modern era, reflecting widespread use of immunomodulatory agents to control inflammation. We performed this study to evaluate the relationships between medical regimens and cytomegalovirus detection rates among patients with inflammatory bowel disease. We searched our database for all patients with established inflammatory bowel disease and severe flares diagnosed from 2002 to 2017. Patients maintained with corticosteroid therapy were considered to be corticosteroid-dependent and those treated with other agents were classified as corticosteroid-independent, provided they had not received corticosteroids within 6 months of colonoscopy. Biopsy samples were reviewed for viral inclusions and subjected to cytomegalovirus immunohistochemistry, and rates of viral detection were compared between groups. There were 135 corticosteroid-dependent patients; most had ulcerative colitis flares occurring during the 2002–2009 period. Patients with ulcerative colitis and Crohn disease were equally represented in the corticosteroid-independent group ($n = 133$) and most were evaluated for disease flares during the 2010–2017 interval. Cytomegalovirus was detected in 13 (8%) cases; 9 (69%) were diagnosed from 2002 to 2009 and all were obtained from corticosteroid-dependent patients ($p = < 0.001$). We conclude that rates of cytomegalovirus-related enterocolitis are declining among inflammatory bowel disease patients, reflecting a shift away from corticosteroid-based maintenance therapy in favor of more effective agents that do not promote viral reactivation.

Introduction

Cytomegalovirus is a member of the human herpesvirus family with a high prevalence of infection among adult patients [1, 2]. Although primary infection often produces no, or only mild symptoms, reactivation of latent disease is a common cause of systemic illness among immunocompromised patients. Risk is highest among patients with HIV/AIDS and transplant recipients but is also increased among individuals receiving systemic chemotherapy, immunosuppressive treatment for immune-mediated diseases, those who are pregnant, and elderly patients with no other apparent causes of immunodeficiency [3–6]. Reactivation of cytomegalovirus in the gastrointestinal tract is a well-known complication of immunosuppressive therapy among patients with inflammatory bowel disease, in which case symptoms and histologic features often mimic a flare of disease activity. The incidence of cytomegalovirus reactivation in this setting ranges from 4.5% to 16%, although some data suggest that up to 25% of patients with chronic, medically refractory colitis harbor cytomegalovirus in inflamed mucosae [7–14]. Distinction between cytomegalovirus-related inflammation and a flare of inflammatory bowel disease is clinically important. Failure to recognize

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cytomegalovirus as the cause of symptoms generally leads clinicians to conclude that the level of immunosuppression is inadequate. Increased immunosuppression can worsen cytomegalovirus-induced inflammation, causing tissue necrosis and even bowel perforation. Unfortunately, detection of viral inclusions in the setting of inflammatory bowel disease is challenging because infected cells are often scarce or obscured by mucosal inflammation [15, 16]. Thus, many pathologists routinely perform immunohistochemical stains to facilitate cytomegalovirus detection in samples from patients with inflammatory bowel disease.

Although it is our practice to obtain cytomegalovirus immunostains whenever biopsy samples from treated inflammatory bowel disease patients show chronic active enterocolitis and ulcers, we have noted a striking decrease in the number of immunopositive cases over the past several years. We believe this observation is related to decreased use of corticosteroids as maintenance therapy for these patients. We performed the current study to determine the frequency of cytomegalovirus detection among inflammatory bowel disease patients treated in an era of widespread use of immunomodulatory and biologic agents for both ulcerative colitis and Crohn disease and compared the results with historical data. We identified colonic biopsy samples from inflammatory bowel disease patients with chronic active colitis and ulcers treated during a 15-year period (2002–2017) and evaluated them for the presence of cytomegalovirus using a combination of histologic review and immunohistochemistry. Rates of viral detection among patients maintained with corticosteroid therapy were compared with those of patients treated with a combination of aminosalicylates, biologic agents, and immunomodulatory drugs, in order to determine whether modern therapies have affected rates of cytomegalovirus reactivation in patients with inflammatory bowel disease.

Materials and methods

Case selection

We retrospectively reviewed the files of the Department of Pathology and Laboratory Medicine at Weill Cornell Medicine to identify all cases of chronic colitis and ulcers diagnosed from 2002 to 2017, inclusively. Electronic medical records of 579 patients were reviewed and information regarding the nature of disease, duration of colitis, type and duration of medical treatment, and colectomy status was recorded. Untreated patients with newly diagnosed inflammatory bowel disease, patients with prior surgery, and individuals with chronic colitis due to other causes were excluded. Patients were classified as either corticosteroid-dependent or corticosteroid-independent. Corticosteroid-dependent patients received corticosteroids in their maintenance regimens and were regularly taking corticosteroids at the time of colonoscopy. Patients were considered to be corticosteroid-independent when they were maintained with various combinations of other drugs, such as aminosalicylates, immunomodulators, and biologic agents. Patients who had recently (<6 months) transitioned from corticosteroid-based regimens to other agents, or received corticosteroids for symptom flares within 6 months of colonoscopy, were excluded from the study in order to limit potential confounders.

Histopathologic evaluation

Routinely processed, hematoxylin and eosin (H&E)-stained tissue sections from all cases were evaluated for cytomegalovirus inclusions. Samples with large atypical cells that showed viral cytopathic changes (e.g. nucleomegaly, granular cytoplasmic inclusions, and/or nuclear inclusions) were classified as positive for cytomegalovirus and confirmed with immunohistochemical stains (DAKO cytomegalovirus M0854 Santa Clara, CA) using standard techniques and appropriate controls. All other samples with severely active chronic colitis and ulcers were also subjected to immunohistochemistry. Viral detection rates among corticosteroid-dependent patients were compared with those of patients receiving other therapeutic agents. Fisher's exact test was used to test the associations among categorical variables. Firth's penalized likelihood method was used for multivariable logistic regression. All analyses were performed using statistical software SAS Version 9.4 (SAS Institute, Cary, NC). A p value < 0.05 was considered to be statistically significant.

Results

The study group consisted of 298 biopsy samples from 268 patients, including 169 with ulcerative colitis and 99 with Crohn disease. Their clinical features are summarized in Table 1. Colonic biopsy samples were obtained from 268 patients, including 115 men and 153 women. There were 135 patients in the corticosteroid-dependent group and 133 corticosteroid-independent patients. Individuals in the latter group were maintained with aminosalicylates, biologic agents (e.g. infliximab, adalimumab), immunomodulatory drugs (e.g. methotrexate, mercaptopurine), or a combination of multiple agents. Most (78%) patients in the corticosteroid-dependent group had underlying ulcerative colitis, whereas similar numbers of patients in the corticosteroid-independent group had

Table 1 Clinicopathologic features of corticosteroid-dependent inflammatory bowel disease patients and those maintained with other therapies

	Corticosteroid-dependent patients ($N = 135$)	Corticosteroid-independent patients ($N = 133$)
Age (mean)	46 years	43 years
Male/female ratio	52/83	63/70
Diagnosis		
Crohn disease	30 (22%)	69 (52%)
Ulcerative colitis	105 (78%)	64 (48%)
Tissue biopsy collection		
2002–2009	102 (76%)	2 (2%)
2010–2017	33 (24%)	131 (98%)
Cytomegalovirus detection	12 (9%)	0 (0%)
2002–2009	8 (67%)	0 (0%)
2010–2017	4 (33%)	0 (0%)
Subsequent colectomy	38 (28%)	20 (15%)

ulcerative colitis (48%) and Crohn disease (52%). Most patients in both groups were middle-aged adults with a slight female predominance. Not surprisingly, 76% of corticosteroid-dependent patients were examined prior to 2010 and most (83%) had underlying ulcerative colitis. Patients with ulcerative colitis (58%) and Crohn disease (75%) were more likely to be managed with immunomodulatory therapies and/or biologic agents after 2010. Treatment regimens for patients in the corticosteroid-independent group included aminosalicylates alone ($n = 43$), immunomodulatory agents alone ($n = 17$), biologic agents alone ($n = 44$), and a combination of immunomodulatory and biologic agents ($n = 29$). Thirty-eight patients (28%) in the corticosteroid-dependent group and 20 patients (15%) in the corticosteroid-independent group ultimately underwent a colectomy procedure ($p = 0.01$).

Cytomegalovirus inclusions were detected in 13 (8%) biopsy samples from 12 patients. They were detected in routinely stained sections in 12 samples and one sample contained enlarged, atypical endothelial cells suspicious for cytomegalovirus infection. All of these cases were confirmed with cytomegalovirus immunohistochemistry (Fig. 1). The number of inclusions present in each case ranged from a single cell to more than ten infected cells per sample. Patients with cytomegalovirus reactivation were older than cytomegalovirus-negative individuals (mean: 63 years versus 44 years, $p = < 0.01$) and tended to be women, although the sex difference was not significant ($p = 0.08$). Ten (83%) patients with viral inclusions in their biopsy samples had ulcerative colitis; the duration of disease at the time of detection ranged from several months to 30 years. All patients with detectable cytomegalovirus were corticosteroid-dependent and had received corticosteroid therapy within 2 months of the endoscopic procedure. Serologic studies were performed for three patients with biopsy-proven cytomegalovirus infection; all had elevated anti-cytomegalovirus IgG titers, but anti-cytomegalovirus IgM was not detected in any patient (Table 2). Quantitative PCR for cytomegalovirus was performed on peripheral blood from six patients with cytomegalovirus-positive biopsy samples. Viral loads ranged from <200 copies/mL to 63,600 copies/mL; there was no correlation between the viral load and density of viral inclusions in colonic samples. Seven (58%) patients with cytomegalovirus reactivation eventually underwent colectomy, which was a significantly higher rate than that of the rest of the cohort (22%, $p < 0.05$), and higher than that of corticosteroid-dependent patients with cytomegalovirus-negative colonic samples (25%, $p = 0.04$). Multivariable logistic regression demonstrated that cytomegalovirus detection was significantly associated with corticosteroid use, independent of age and sex ($p = 0.03$). A sub-analysis limited to ulcerative colitis patients revealed a significant relationship between corticosteroid use and cytomegalovirus detection ($p = 0.014$). Of note, most (69%) cytomegalovirus-positive cases were obtained during the 2002–2009 period. Rates of viral detection declined from 7.4% (2002–2009) to 2.2% (2010–2017) during the study period.

Discussion

The purpose of this study was to systematically evaluate relationships between treatment regimens and cytomegalovirus detection rates among patients with established inflammatory bowel disease and to re-evaluate the role of cytomegalovirus immunohistochemistry in an era of immunomodulatory and biologic agents. We performed cytomegalovirus immunohistochemistry on 298 samples from 268 patients with established inflammatory bowel disease and ulcers despite medical therapy. We found that cytomegalovirus detection is strongly associated with recent corticosteroid use ($p < 0.001$), particularly among patients with ulcerative colitis ($p = 0.02$), and these relationships persist following multivariable logistic regression analysis. We also found that rates of cytomegalovirus detection have decreased from 7.4% to 2.2% over a 15-year period, corresponding with an overall decline in corticosteroid use among both ulcerative colitis and Crohn disease patients. These findings suggest that cytomegalovirus is an unlikely cause of colitic symptoms among inflammatory bowel disease patients who have not recently received corticosteroid therapy. Routine use of cytomegalovirus immunohistochemical stains in all inflammatory bowel disease patients

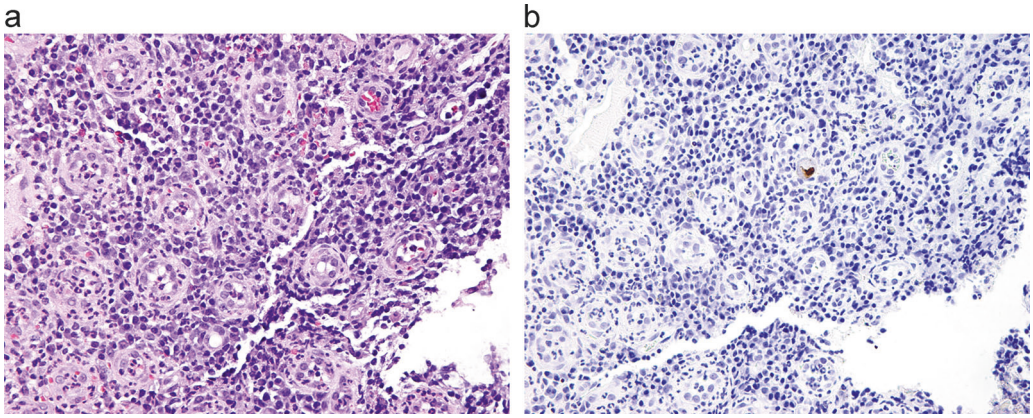

Fig. 1 Twelve patients had histologically evident, immunohistochemically confirmed cytomegalovirus in their colonic biopsy samples. One biopsy sample showed ulcers and inflamed granulation tissue without detectable cytomegalovirus inclusions (**a**). However, occasional enlarged endothelial cells strong nuclear immunostaining for cytomegalovirus in the same sample (**b**)

Table 2 Results of serologic studies from patients with cytomegalovirus inclusions in colonic biopsy samples

Patient	Viral load (DNA copies/mL)	Serologic findings
1	-	-
2	-	-
3	63,600	-
4	-	-
5	-	-
6	-	-
7	<200	-
8	900	IgG+/IgM−
9	-	IgG+/IgM−
10	<200	IgG+/IgM−
11	1818	-
12	<137	-

with medically refractory disease may be unnecessary in the modern era.

Cytomegalovirus reactivation is a well-known complication of inflammatory bowel disease treatment and distinguishing it from an exacerbation of existing inflammatory bowel disease is clinically important. Additional immunosuppressive therapy usually worsens cytomegalovirus-related intestinal injury and can result in toxic megacolon, perforation, or even death [17–19]. Reported frequencies of cytomegalovirus reactivation in the setting of inflammatory bowel disease vary depending on the method of viral detection, nature of underlying inflammatory bowel disease, severity of inflammatory activity, and type of therapeutic intervention [7, 8, 18, 20, 21]. The diagnosis of cytomegalovirus-related colonic inflammation largely rests on histologic identification of viral inclusions in tissue samples, which can be enhanced with immunohistochemistry. Serologic studies are of limited value because they do not always distinguish remote viral exposure from active infection. Assays that detect cytomegalovirus DNA by PCR are frequently performed to detect symptomatic cytomegalovirus infection but do not necessarily correlate with the abundance of inclusions detected in mucosal samples [22].

Reactivation of cytomegalovirus is more likely among patients with inflammatory bowel disease who are further immunocompromised by poor nutrition, medications, or immune dysfunction. The virus demonstrates tropism for sites of inflammation and, thus, reactivation risk is often related to extent and severity of inflammation. Overexpression of tumor necrosis factor (TNF) promotes disease activity and plays a role in activating latent virus through interactions with other proinflammatory cytokines [23–28]. Presumably, circulating monocytes and dendritic cells that contain latent cytomegalovirus migrate to sites of inflammation, where they elaborate IL-6, TNF-α, IL-1β, and other cytokines [23, 29–31]. Yi et al. utilized a combination of serum PCR, tissue samples, and antibody titers to evaluate the prevalence of, and clinical risk factors for, cytomegalovirus infection among 189 ulcerative colitis patients and 37 with Crohn disease; they found elevated serum DNA levels were associated with severe disease activity ($p = 0.05$) and recent corticosteroid therapy ($p = 0.04$) [7]. Others have also found a higher prevalence of cytomegalovirus reactivation among patients with refractory ulcerative colitis, likely reflecting the tendency for corticosteroids to directly activate viral replication and suppress antiviral T-cell specific function [8, 12, 13, 18, 32–35].

Therapeutic management of inflammatory bowel disease has drastically changed over the last several years. Corticosteroids are mostly reserved for induction of remission but are not widely used for maintenance therapy due to a variety of adverse effects [36]. Single immunomodulatory and/or biologic agents, or combinations of drugs, are highly effective for maintaining remission and

preventing relapse among both ulcerative colitis and Crohn disease patients. Azathioprine, 6-mercaptopurine, and methotrexate reduce recurrence rates of both Crohn disease and ulcerative colitis among patients with mild to moderate disease [37–41]. Biological agents, including infliximab, adalimumab, and certolizumab pegol, inhibit TNF-α-mediated inflammation and are effective treatment options for patients with moderate-to-severe ulcerative colitis and Crohn disease [42–44]. Cyclophosphamide, cyclosporine, and tacrolimus can also be used in selected patients.

The reported prevalence of infectious complications among patients receiving immunomodulatory and biologic therapies varies considerably. Early randomized clinical trials reported no increased risk for serious infections among patients treated with TNF-α inhibitors, yet subsequent studies have reported infection rates of 2.8–4% among patients receiving biologic agents [45–50]. Commonly reported infections include hepatitis B virus, herpes zoster virus, and tuberculosis reactivation [51–54]. Overall, the odds of developing an infection while receiving biologic agents are increased (19%), but numerous placebo-controlled trials have failed to consistently demonstrate a risk of serious infection [55]. Cytomegalovirus reactivation is less commonly associated with newer agents than corticosteroid therapy [56–60]. McCurdy et al. performed a retrospective case-control study and found that medically refractory inflammatory bowel disease (OR 3.69), corticosteroid therapy (OR 2.95), and immunomodulatory drug therapy (OR 1.86) were significantly associated with cytomegalovirus-related colitis whereas TNF-α antagonists did not cause cytomegalovirus-related morbidity [20]. Doménech et al. performed a prospective study evaluating 114 patients with ulcerative colitis and identified cytomegalovirus colitis in only patients who had received high doses of corticosteroids within 7–10 days of the diagnostic procedure [8].

In summary, our findings indicate that the rate of cytomegalovirus detection among patients with colitic symptoms is declining among patients with established inflammatory bowel disease. This trend likely reflects a shift away from maintenance therapy with corticosteroids toward more effective biologic and immunomodulatory agents, particularly among patients with ulcerative colitis. Immunohistochemical stains for cytomegalovirus are of limited value when applied to samples obtained from patients with inflammatory bowel disease who have not been recently exposed to corticosteroid therapy, especially when they are maintained with biologic agents.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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