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Absence of focally enhanced gastritis in macaques with idiopathic colitis

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Abstract

Aim—Focally enhanced gastritis has been described in association with Crohn's disease and ulcerative colitis, but is rare in the general population. The study aim was to test whether idiopathic colitis in macaques was associated with any characteristic changes of the gastric mucosa resembling similar changes in humans.

Methods—Presence or absence of idiopathic colitis was established by gross and microscopic examination of the colons of rhesus macaques (*Macaca mulatta)*, which died at the Oregon National Primate Research Center. Gastric tissue specimens were compared between a case population of 26 macaques with idiopathic colitis and a control population of 21 macaques without colitis. The specimens were histologically assessed by two independent pathologists blinded to the presence or absence of idiopathic colitis. Differences between cases and controls were compared using a two-sided Fisher's exact test.

Results—Of the 26 case macaques with colitis, 11 animals (42%) harbored signs of chronic gastritis. Of the 21 control macaques without colitis, 9 animals (43%) harbored signs of chronic gastritis, p=1.0000. Of all animals with gastritis, 1/11 animals with colitis and 2/9 control animals showed rare active gastritis as evidenced by the presence of neutrophils, p=0.5658. Lymphocytic infiltrates of the gastric mucosa were seen in 4/11 colitis cases and 0/9 controls, p=0.0942. No gastric specimens with focally enhanced gastritis were found among any of the case or control animals.

Conclusions—Unlike chronic inflammatory bowel disease in humans, idiopathic colitis in macaques is not associated with focally enhanced gastritis or any other type of specific gastritis.

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STATEMENT OF INTEREST

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Keywords

etiology of inflammatory bowel disease; focally enhanced gastritis; gastritis; idiopathic chronic colitis of macaques; infectious organisms in inflammatory bowel disease; rhesus macaques (*Macaca mulatta)*

INTRODUCTION

Focally enhanced gastritis has been reported to be frequently associated with Crohn's disease and ulcerative colitis, but is rare in the general population (1–3). Focally enhanced gastritis is typified by small collections of lymphocytes and histiocytes surrounding a group of a few gastric glands, often associated with infiltrates of neutrophils into the glands in otherwise unremarkable gastric mucosa (1,4,5). Macaques held in captivity frequently develop an idiopathic chronic colitis of unknown etiology that has served as animal model for inflammatory bowel disease of humans (6–9). Chronic diarrhea of macaques leads to dehydration and progressive weight loss requiring frequent veterinary intervention. Multiple previous attempts at isolating specific viral, bacterial, or parasitological pathogens have failed, and in general the idiopathic chronic colitis does not respond to treatment with antibiotics (10–11). This type of colitis in monkeys may provide clues about the etiology of chronic inflammatory bowel disease in humans. The aim of the present study was to test whether idiopathic colitis in monkeys was associated with any characteristic changes of the gastric mucosa.

METHODS

The present study deals with adult rhesus macaques (*Macaca mulatta*) from the Oregon National Primate Research Center (ONPRC). The monkeys were kept in multiple-occupancy cages of the center's outdoor facility. They were fed a standard commercial food (Lab Fiber-Balanced Monkey Jumbo 5000, Brentwood, MO) with supplemental fruit and water ad libitum. Rhesus monkeys with or without idiopathic chronic colitis may have been housed in the same area. A complete necropsy with histological examination of major organs was performed by board-certified veterinary pathologists of the Pathology Services Unit in all monkeys, which died at the Oregon National Primate Research Center. The study protocol was exempt from approval by the institutional review board, because all necropsies and histological examinations were performed as part of the routine clinical care. The presence or absence of idiopathic colitis was established by gross and microscopic examination of the colons of all animals.

Gastric tissue specimens were selected from 26 animals with idiopathic chronic colitis, while 21animals without colitis served as controls. After fixation in 10% neutral buffered formalin, gastric and colonic tissues were dehydrated and embedded in paraffin. Sections were cut at 5 microns and stained with hematoxylin and eosin. The randomized slides of gastric tissue specimens from the 47 individual monkeys were independently assessed by two board-certified pathologists with subspecialty training in gastrointestinal pathology. The two pathologists were blinded to the presence or absence of idiopathic colitis in the individual animals. Disagreement between the two pathologists was resolved by joint re-evaluation of controversial specimens.

Focally enhanced gastritis has been reported in up to 70% of patients with Crohn's disease (1), 20% of patients with ulcerative colitis (2), and less than 5% of subjects without idiopathic inflammatory bowel disease (1–2). To establish a statistically significant difference between 70% and 5% with an alpha-error < 5% and a beta-error < 20%, samples

size of 10 case and control animals are needed. To establish a statistically significant difference between 45% (= (70+20%)/2) and 5%, sample sizes of 22 case and control animals are needed. Differences in the frequency of gastritis between cases and controls were compared using a two-sided Fisher's exact test.

RESULTS

The two panels of Figure 1 depict the macroscopic and microscopic appearance of the colon from a rhesus monkey with idiopathic chronic colitis. The entire colon was affected by severe inflammatory changes resulting in a thickened and flaccid colonic wall. The mucosa was markedly thickened, erythematous, and covered by multiple erosions (Figure 1a). Microscopically, the chronic colitis was characterized by lympho-plasmacytic infiltration, crypt epithelial hyperplasia, goblet cell depletion, and the presence of multifocal crypt abscesses (Figure 1b). Figure 2 shows for comparison the macroscopic and microscopic appearance of a normal colon in a rhesus monkey.

Of the 26 case monkeys who died with idiopathic colitis, 11 animals (42%) harbored signs of chronic gastritis, which was diagnosed in the presence of increased lymphocytes and plasma cells within the lamina propria with or without prominent secondary lymphoid follicle formation. Of the 21 control monkeys who died without idiopathic colitis, 9 animals (43%) harbored signs of chronic gastritis, p=1.0000. The top panel of Figure 3 depicts an example of the most commonly seen chronic gastritis without active inflammation. Of all animals with gastritis, 1/11 animals with colitis and 2/9 control animals showed rare active gastritis as evidenced by the presence of neutrophils, p=0.5658. As illustrated by the bottom panel of Figure 3, active gastritis was characterized by intraepithelial and/or intraluminal neutrophils, marked in the figure by arrows. Gastric intraepithelial lymphocytes (virtually absent in normal subjects) were seen in 4/11 colitis cases and 0/9 controls, p=0.0942. Figure 4 shows an example of such "lymphocytic gastritis" in a background of increased chronic inflammation; there are foveolae studded with intraepithelial lymphocytes (between the thin arrows). A similar feature is seen on the surface epithelium (indicated by the larger arrow).

Focally enhanced gastritis was not identified in any of the case or control animals.

DISCUSSION

The occurrence of Crohn's disease and ulcerative colitis in humans has been associated with a particular histological finding, commonly referred to as "focally enhanced gastritis." The aim of the present study was to test whether the idiopathic chronic colitis of rhesus monkeys was similarly characterized by the presence of a specific type of gastritis. No evidence of focally enhanced gastritis was found in any animal affected by chronic colitis, as well as any of the control animals. Moreover, other types of gastritis also failed to show any significant relationship with the occurrence of idiopathic chronic colitis of rhesus macaques. The findings of the present study suggest that focally enhanced gastritis may be a unique feature of inflammatory bowel disease in humans.

Monkeys, like all other mammals, can readily form granulomas and do so in the face of persistent pathogens and antigens, which typically incite granulomas in humans, such as *Mycobacterium tuberculosis*, foreign bodies, and fungi. Granulomas as characteristic features of Crohn's disease, however, were not observed in any specimens of the colonic or gastric mucosa from rhesus monkeys affected with idiopathic chronic colitis. It is difficult to assess to what extent idiopathic chronic colitis in rhesus monkeys represents a valid model of human inflammatory bowel disease. It is conceivable that conditions of living in captivity are partly responsible for the lesions observed either at the level of the colon or at the level

of the stomach. The animals live in social housing with close contact and easy transmission of pathogens. They live socially in the wild but are not as geographically restricted as at the ONPRC, with different conditions for environmental contamination. Since animals are kept in more fixed social groups, there may be variable social stress compared with free-ranging animals. Although macaques are outbred and significantly more genetically diverse than rodent lab animals, the ONPRC population is still potentially more genetically limited than wild populations. In the ONPRC, animals with idiopathic chronic colitis are medically treated, allowing for persistence of chronic disease. In the wild, there would be more selection against affected animals. Although the monkeys are feed a nutritionally complete, balanced, and defined diet, it is obviously not the same as a wild diet. During their lifetime, the animals may also be treated with a variety of antimicrobials that affect their gut flora in ways that wild animals would not experience.

There are several potential limitations to our study. In has been shown that in humans focally enhanced gastritis can be masked by the simultaneous presence of *H. pylori*-gastritis (12). Similarly, in our population of rhesus macaques, the presence of focally enhanced gastritis may have been obscured by the dense chronic inflammation, which may be associated with the *Helicobacter* spp. that naturally infect a large proportion of these animals (13,14). However, this seems unlikely, as we failed to detect traces of focally enhanced gastritis also in animals without signs of chronic gastritis. One study reported that more than 70% of patients with Crohn's disease harbor focally enhanced gastritis (1), but other studies have not been able to confirm this elevated prevalence. Since chronic gastritis was present in only 42% of our macaques with colitis, if focally enhanced gastritis were truly associated with idiopathic chronic colitis in rhesus macaques, we should have been able to see some instances of focally enhanced gastritis among our colitis cases without gastritis. Gastritis associated with idiopathic chronic colitis in rhesus macaques could also present with features different from those of focally enhanced gastritis in humans. Lymphocytic infiltrates of the gastric mucosa were seen more frequently in colitis cases than controls, but this difference failed to reach statistical significance. The overall prevalence of intraepithelial lymphocytosis among the colitis cases was much less than the prevalence of focally enhanced gastritis reported among subjects with Crohn's disease. Yet, there remains a remote possibility that a larger sample size may have revealed a statistically significant difference.

The histopathologic findings in the gastric biopsies described in association with human inflammatory bowel disease include granuloma formation, focally enhanced gastritis, and nonspecific gastritis (1,4,5). These histopathological features are distinct from other types of gastritis (15–17). The etiology of focally enhanced gastritis is unknown, although the infiltrates of neutrophils surrounding the collections of lymphocytes and histiocytes may suggest a bacterial or other infectious etiology. Such infection could represent an epiphenomenon of inflammatory bowel disease or could be intimately linked to its etiopathogenesis and potentially open a window to study its yet unknown etiology. The results of the present study suggest that focally enhanced gastritis does not occur in the idiopathic chronic colitis of macaques and may represent a unique feature of human inflammatory bowel disease.

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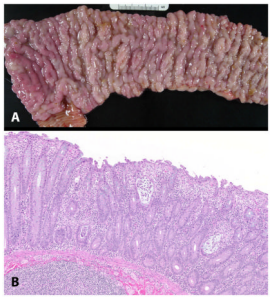

Figure 1.
A: Macroscopic appearance of idiopathic chronic colitis at the ileocolonic junction of rhesus macaques; the scale bar is in mm. B: Microscopic appearance of idiopathic chronic colitis; original magnification is 100 ×.

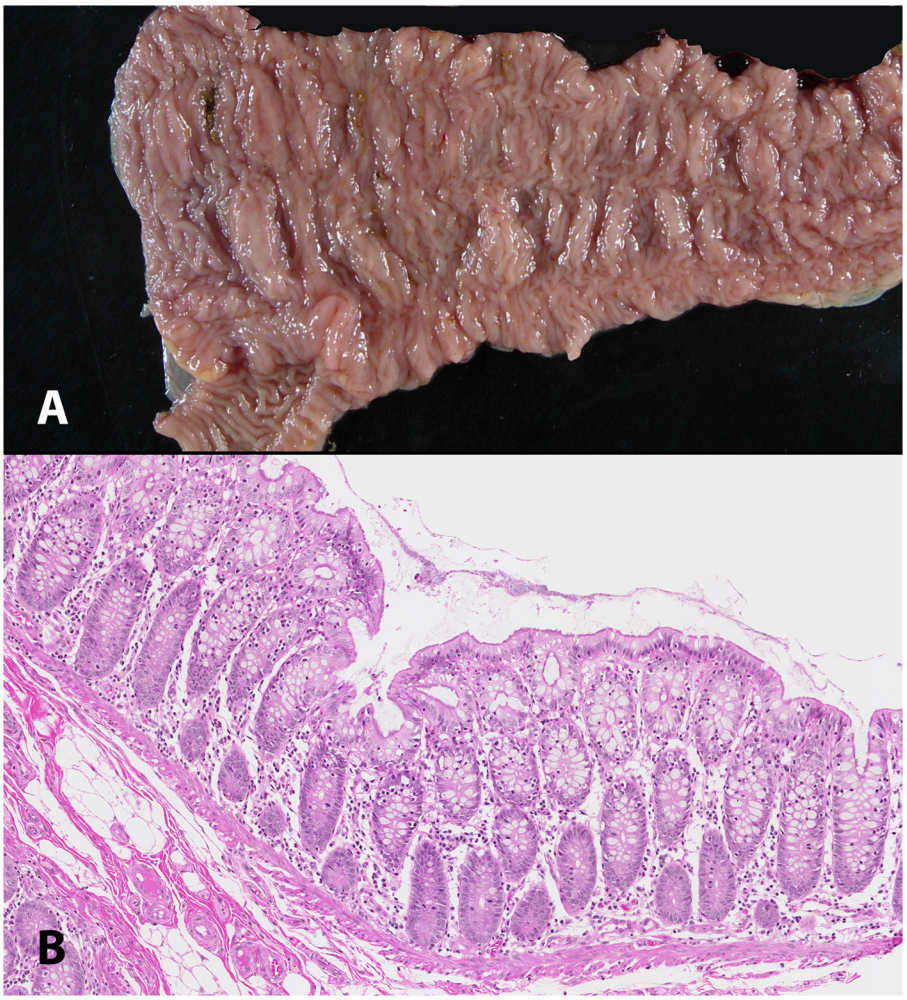

Figure 2.
A: Macroscopic appearance of normal ileocolonic junction from a rhesus macaque *without* idiopathic chronic colitis. B: Microscopic appearance of normal colonic mucosa from a rhesus macaque; original magnification is 100 ×.

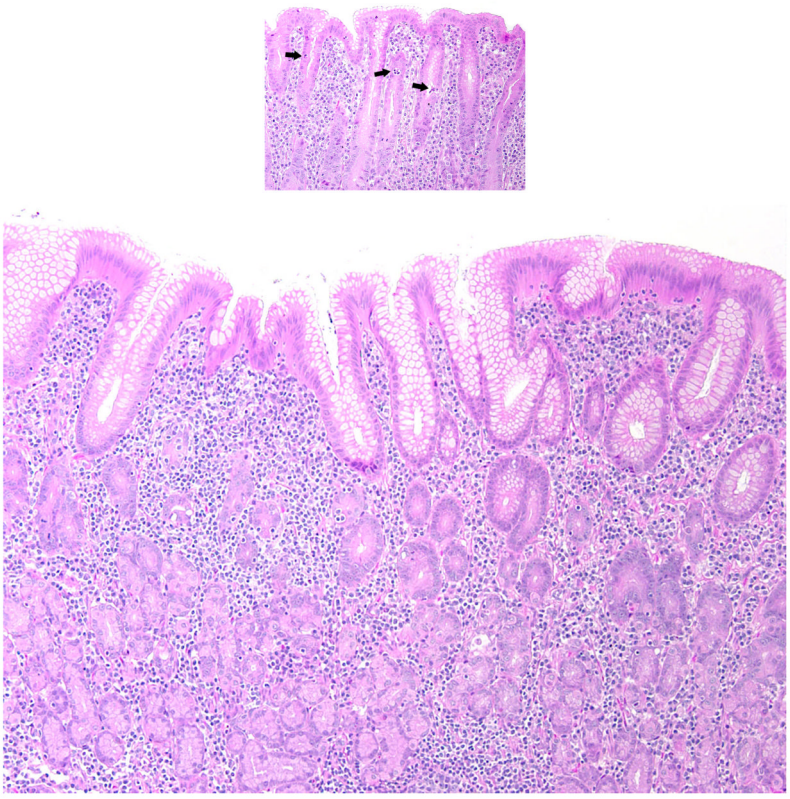

Figure 3.
Top: Chronic gastritis without signs of active inflammation; original magnification is 200 ×. Bottom: Chronic gastritis with infrequent occurrence of active inflammation; the occurrence of neutrophils is indicated by arrows; original magnification is 400 ×.

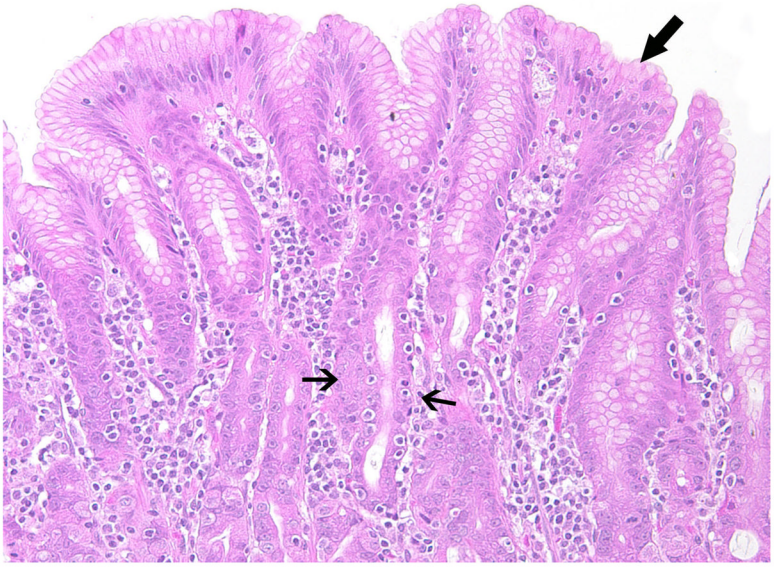

Figure 4.
Lymphocytic gastritis. In a background of increased chronic inflammation, there are foveolae studded with intraepithelial lymphocytes (between the thin arrows). A similar feature is seen on the surface epithelium (indicated by the larger arrow). Original magnification is 400 ×.