A Case of Intestinal Mucormycosis in a Common Marmoset (*Callithrix jacchus*)

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ABSTRACT. A 3-year-old female common marmoset was euthanized because of wasting. Grossly, a perforating lesion was present in the jejunum and hemorrhagic lesions in the cecum and colon. Histopathologically, these gross lesions were a perforated jejunal ulcer and necrotizing colitis, respectively. Necrotizing colitis was characterized by extensive mucosal necrosis along with numerous ribbon-shaped aseptate hyphae. These aseptate hyaline hyphae were positively stained with PAS and GMS, and reacted immunohistochemically with the antibody against the family *Mucoraceae*. This case was diagnosed as intestinal mucormycosis. This is the first report on mucormycosis in a common marmoset.
KEY WORDS: common marmoset, intestinal mucormycosis, wasting.

Organisms of the order *Mucorales*, class *Zygomycetes*, are widely distributed in nature as air-borne saprophytic fungus. These fungi have a worldwide distribution and have been isolated from a variety of food items [1]. The fungal spores are ubiquitous in the enviroment and could therefore be acquired through inhalation, traumatic inoculation or via the skin or mucosa. Zygomycosis is the generic term for a fungal infection caused by a fungus of the class *Zygomycetes*. An infection caused by a fungus of the order *Mucorales* (*Absidia*, *Mucor*, *Rhizomucor*, *Rhizopus* or *Saksenaea* spp.) is called mucormycosis. Mucormycosis is the third most frequent invasive fungal infection, after aspergillosis and candidiasis [4]. Mucormycosis is an opportunistic fungal infection in the compromised host, and a deep-seated mycosis, distinguished by multiple clinical disease types: rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated, and miscellaneous. In humans, the most common patterns of infection are the rhinocerebral, pulmonary, cutaneous, and disseminated types [15]. The gastrointestinal type is rare, but mortality is high [16]. In non-human primates, there are only seven reports of natural infectious mucormycosis in the literature; these reports include the rhino-orbital type in a rhesus monkey (*Macaca mulatta*) [10], the cutaneous type in a rhesus monkey [2], the gastrointestinal type in two rhesus monkeys [5, 6] and two spider monkeys (*Ateles hybridus*) [11], and the generalized type in a mandrill (*Mandrillus sphinx*) [9] and a golden-bellied mangabey (*Cercocebus galeritus chrysogaster*, Lydekker) [8]. However, there have been no reports of mucormycosis in common marmosets (*Callithrix jacchus*).

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Here, we report the first case of intestinal mucormycosis and describe the histopathological, immunohistochemical and electron microscopic findings in a common marmoset.

A 3-year-old female common marmoset, that had been bred in an another institution, was sent to our laboratory because of wasting, and then euthanized. The weight of the animal had decreased from 270 g to 206 g in the past 2 weeks, and it had become weak. However, except for emaciation, clinical symptoms such as diarrhea were not observed. Before admission to our laboratory, this animal was fed the pelleted 'CMS-1M' diet (CLEA Japan, Tokyo, Japan) in the institution, and had no history of experimental or medical treatment, including antibiotic administration. The cause of weakening was not explained in information sent previously from the institution.

Necropsy showed atrophied visceral and subcutaneous adipose tissues, a perforating lesion in the lower jejunum, hemorrhagic lesions in the cecum and colon, and enlargement of pancreaticoduodenal and ileocecal lymph nodes. A perforation about 2 mm in diameter was present in the lower jejunum on the opposite side of the mesenteric attachment site. Slight leakage of intestinal contents was observed in the abdominal cavity. Adhesion of the perforating lesion site and the surrounding tissue was not found. No other gross lesions were observed.

For light microscopy, the intestines, liver, pancreas, lymph nodes, thymus and spleen were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) or Grocott-Gomori methenamine silver (GMS).

For immunohistochemical analysis, an antibody against *Rhizopus arrhizus* (WSSA-RA-1: monoclonal mouse anti-*Rhizopus arrhizus* antibody; AbD Serotec, Oxford, UK, dilution 1:50) was used. This antibody specifically recog-

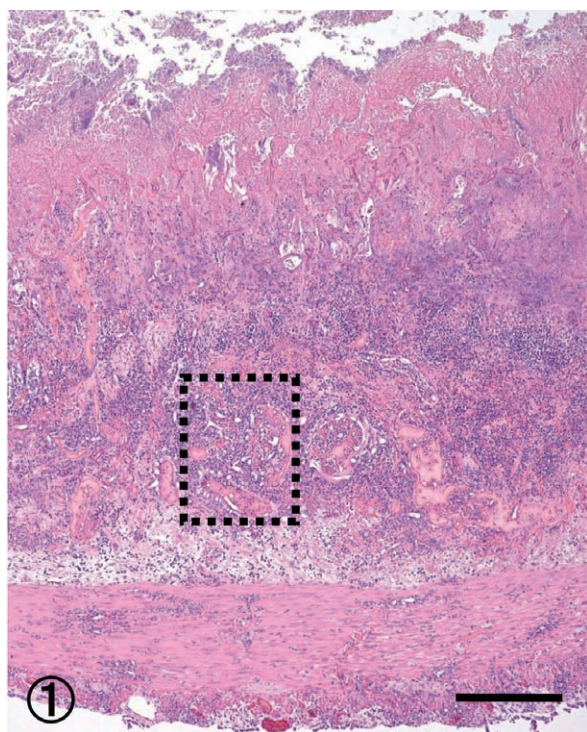Fig. 1. Necrotizing colitis characterized by extensive mucosal necrosis, associated with edema in the submucosa. H&E stain. Scale bar=400 μm.

nizes *Rhizopus arrhizus* and other members of the family *Mucoraceae*, including *Absidia* spp., *Mucor* spp., *Rhizomucor* spp. and *Rhizopus* spp. It reacts strongly with the cytoplasm of the hyphae and also possibly with the walls and septae [7]. Immunohistochemical staining was performed using the Dako Envision kit (Dako Denmark A/S, Glostrup, Denmark). The immunoreaction was visualized by the peroxidase-diaminobenzidine reaction. The sections were finally counterstained with hematoxylin.

For electron microscopy, colonic specimens from formalin-fixed and paraffin-embedded blocks were deparaffinized, fixed in osmium tetraoxide and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a JEOL 100S transmission electron microscope (JEOL Ltd., Tokyo, Japan).

Light microscopy showed a perforated jejunal ulcer associated with perilesional acute peritonitis. The site of the penetrating ulcer was covered with a necrotic layer comprised of fibrin, neutrophils and cellular debris. In the intestinal mucosa, other than the jejunal ulcer, many lymphocytes infiltrated in the lamina propria. In addition, extensive mucosal necrosis, associated with edema in the submucosa, was observed in the cecum and colon (Fig. 1). Numerous ribbon-shaped aseptate hyphae were observed throughout the necrotic mucosa. The hyphae also caused vasculitis in relatively large vessels of the colonic lamina propria and submucosa (Fig. 2-a). Irregularly branching hyphal filaments invaded vessels and the surrounding tissue. These hyphal filaments in both necrotic mucosa and blood vessels were stained pale red by the PAS reaction and brown-black with GMS (Fig. 2-b), and reacted immunohistochemically with the antibody against *Rhizopus arrhizus* (Fig. 2-c). Except for the foci of necrotizing colitis, hyphal forms were not observed at all in the intestine and the mesentery including the jejunal ulcer site. There were no gross lesions in the thymus and spleen, but histopathologically, lymphoid necrosis was present in the thymic cortex. In the spleen, the white pulp had atrophied. In the liver,
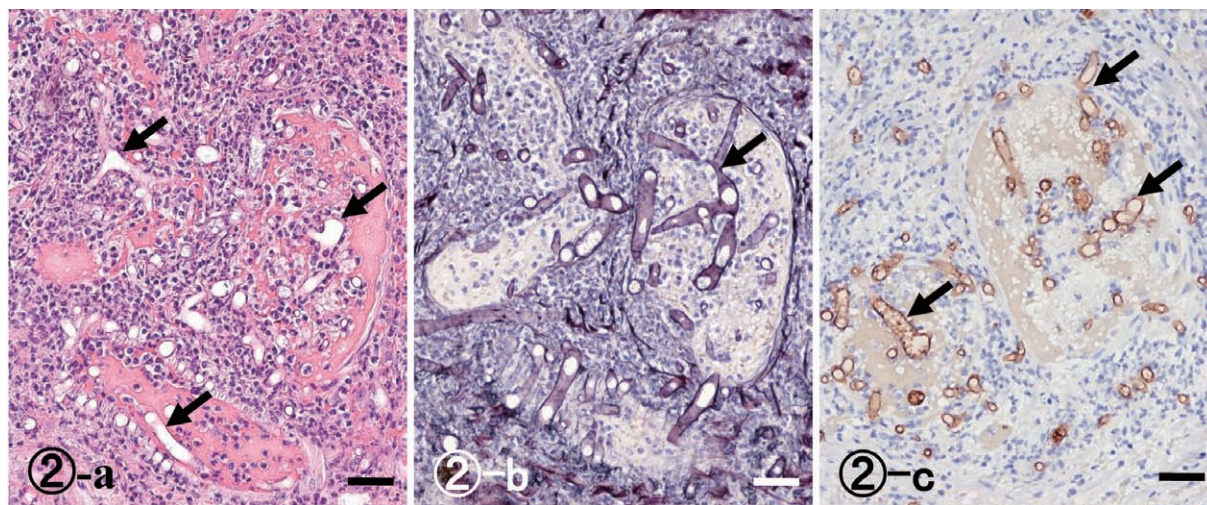

Fig. 2. Photomicrograph of blood vessels of the colonic lamina propria, magnification of Fig. 1. Scale bar=50 μm. a) H&E-stained section of vessels filled with hyphae and inflammatory cells. Pale-stained hyphae resemble bubbles or ribbons in the tissue (arrows). b) GMS-stained section of vessels containing nonseptate fungal hyphae typical of mucormycetes. Wide ribbon-like hyphae with broad-angle branching are seen (arrow). c) Fungal hyphae in vessels reacted immunohistochemically with antibody against the family *Mucoraceae* (arrows).
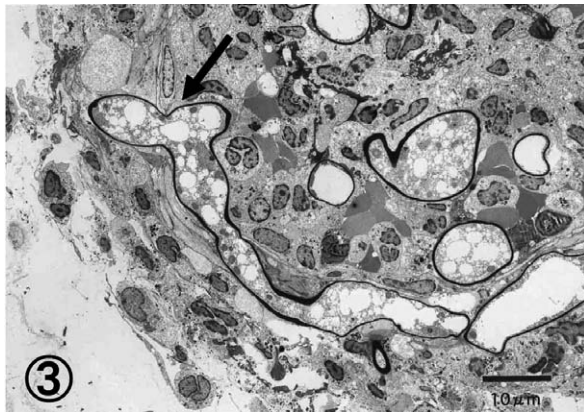
Fig. 3. Transmission electon mycroscopy demonstrates nonseptate coenocytic zygomycete hyphae in a blood vessel of the colonic lamina propria. The fungal hyphae have invaded via the vessel wall (arrow), but marked destruction of the vessel wall by hyphal invasion is not evident. Scale bar=10 μm.

active extramedullary hematopoiesis was observed, but no hepatocellular damage such as fatty changes was present.

Ultrastructual diagnosis clearly revealed that the hyphae showed irregular sequences in the tissue, and irregular branching at wide angles of almost 90 degrees was present (Fig. 3). The hyphal branching morphology in the blood vessel showed that the hyphae had invaded surrounding tissue through the vessel wall. However, severe destruction due to hyphal perforation of the vessel wall was not observed. Electron microscopy made it clear that the hyphae were non-septate, with a variable width of 2 μm to 15 μm, unlike the hyphal morphology of *Aspergillus* with septate hypha (true hypha), or *Candida* with pseudohypha. Moreover, many nuclei within the aseptate hyphae were visible with an electron microscope. Therefore, ultrastructural findings showed that the fungus in this case had non-septate coenocytic hyphae. A diagnosis of intestinal mucormycosis was made based on histopathological, immunohistochemical and electron microscopic pathognomonic findings. To our knowledge, this is the first report of mucormycosis in a common marmoset.

Mucormycosis is an opportunistic fungal infection. In this case, the histological findings which showed lymphocyte depletion of the thymus and spleen suggested that this animal was in an immunocompromised state. Although the cause is uncertain, it is assumed that the host immune status became a key factor in manifestation of mucormycosis.

Mucormycosis of the gastrointestinal tract is rare in both humans and non-human primates. In humans or non-human primates, risk factors, such as malnutrition [12], diarrhea, and gastric ulcers [6] and intestinal ulcers [3, 5, 11, 13] have been associated with the development of gastrointestinal mucormycosis. Thus, gastrointestinal mucormycosis in humans and non-human primates is mostly related to ulcers [14]. However, in this case, no hyphae were observed in the jejunal ulcer site. It is uncertain why intestinal mucormycosis developed in the colon rather than the jejunal ulcer site.

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