ANIMAL MODEL OF HUMAN DISEASE

Bacillary Dysentery, Shigellosis, *Shigella* Dysentery

Animal Model: Monkey Shigellosis or Dysentery

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Biologic Features

Most nonhuman primates are easily infected with shigellae; gorillas [1] seem to be very susceptible, while the Panamanian monkeys appear highly resistant.[2] *Macaca* species also are easily infected.[2] Thus, 22% of a shipment of 4431 rhesus (*M. mulatta*) examined on arrival, although apparently healthy, had stools positive for *Shigella*.[2] Within a few weeks a large percentage had developed clinical shigellosis with diarrhea, probably because of delayed shipment and acclimatization stress. *Shigella* species recovered were *S. flexneri*, *S. dysenteriae*, and *S. sonnei*.[2]

When ill with naturally acquired shigellosis,[3] the rhesus shows weakness and prostration, with a drawn, occasionally edematous face; stools are liquid and contain mucus and blood. If not treated, the animal may die within a few days to 2 weeks. At necropsy, the large intestine is distended, and the mucosa swollen and hemorrhagic with exudate. Histopathology shows acute inflammation in the mucosa with penetration of gut epithelium by Shigellae (Figure 1).

Diagnosis is based on clinical signs and positive culture for *Shigella* in the stool.

Experimental Procedures and Results

To study experimental shigellosis in monkeys, either rhesus [4] or cynomolgus (*M. irus*),[5] one must use monkeys that have passed a quarantine period of 5 to 6 weeks without diarrhea and have stool cultures negative for *Shigella* and *Salmonella* on at least three successive occasions within a week of the beginning of the experiment.[4,5] The challenging dose of 1×10^{10} to 5×10^{10} virulent bacilli (usually *S. flexneri* 2a) suspended

Publication sponsored by the Registry of Comparative Pathology of the Armed Forces Institute of Pathology and supported by Public Health Service Grant RR 00301 from the Division of Research Resources, US Department of Health, Education and Welfare, under the auspices of Universities Associated for Research and Education in Pathology, Inc

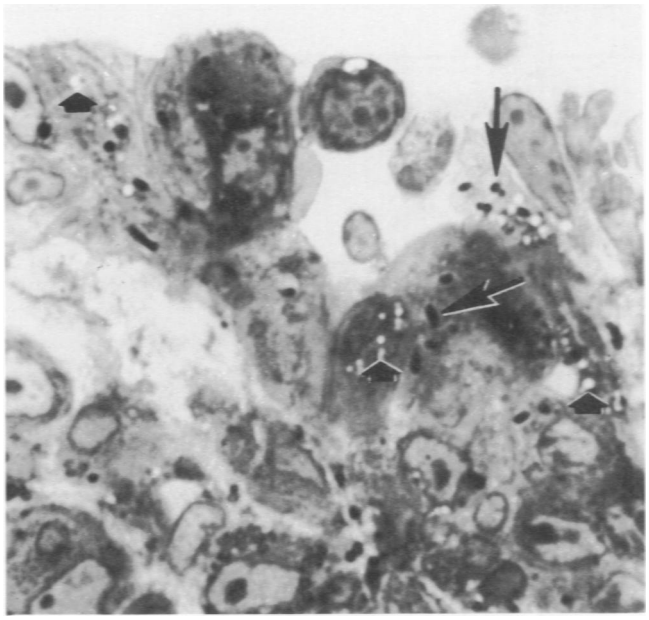

Figure 1—Surface epithelium of colon 24 hours after challenge with shigellae. Rod-shaped bacilli are present in degenerating epithelial cells desquamating into the lumen (*long arrows*). Intraepithelial vacuoles represent abnormal accumulation of lipid droplets (*short arrows*). (× 1600).

in 20 ml of broth is administered by gastric tube. Control monkeys receive 20 ml of sterile broth. There is a variable response with different batches of animals, but the infected animals usually develop clinical signs and symptoms of acute shigellosis 24 to 48 hours after oral challenge. Cultures as well as gross and microscopic observations are made periodically on stool samples from both infected and control monkeys and are repeated when the animals are sacrificed at various times after challenge. At necropsy, bowel contents are grossly examined and cultured. Representative specimens from the jejunum to the sigmoid are immediately removed for fluorescent antibody, histologic, and electron microscopic examinations.[5-7] Gross abnormalities are usually confined to the colon, although gastric lesions are frequent; small intestinal lesions, on the other hand, are uncommon.[8] The colonic contents consist of mucopurulent to mucohemorrhagic exudate. The colonic wall is thickened, and the mucosa injected, granular, and unevenly swollen. At 48 hours, the above changes become severe and often diffusely involve the entire large bowel. Shallow and circumscribed ulcers are commonly covered by purulohemorrhagic exudate.

Histologically,[4-8] the lesions represent an acute colitis; mild infections are characterized by a catarrhal inflammation with a distinct gradation of the inflammatory response, diminishing from the luminal surface to the submucosa which shows little or no reaction. There are flattening, pseudostratification, and accelerated shedding of the epithelium (Figure 1). The more severe forms are similar to a pseudomembranous acute colitis. Ulceration is common (Figure 2). Regardless of the severity of the

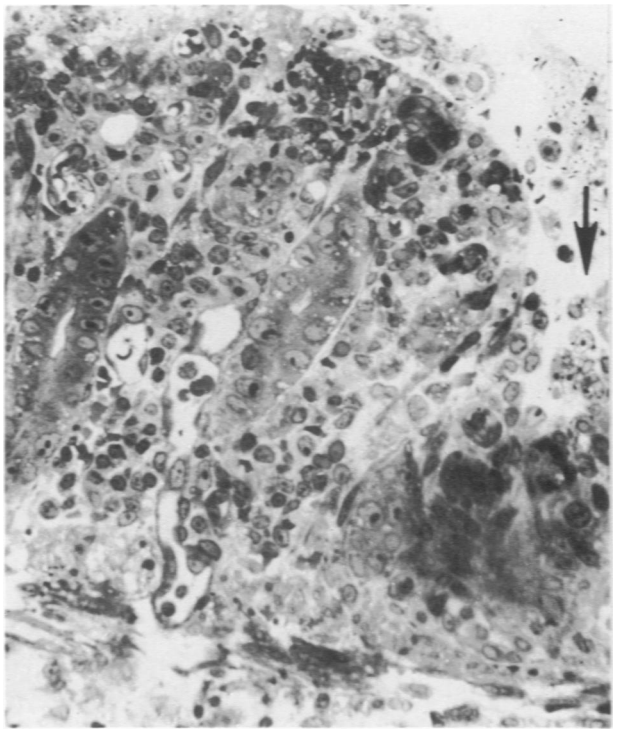

Figure 2—Colonic mucosa 48 hours after challenge with shigellae. A microulcer extends deep into the lamina propria (*arrow*). Surface epithelial cells show extensive degeneration and necrosis. Notice cell debris in the lumen. Subepithelial capillaries are congested and extravasated RBC are present. (× 420)

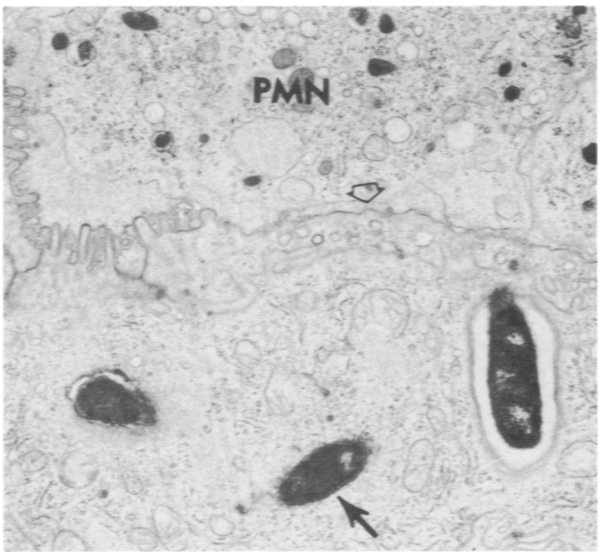

Figure 3—Luminal aspect of crypt epithelial cells. Microvilli at left are preserved, while others are flattened (*short arrow*). A polymorphonuclear leukocyte (*PMN*) in the crypt lumen is in direct contact with altered microvilli. Intraepithelial bacilli are enclosed by a single or double membrane. Occasionally they lack such membranes and lie free in the cytoplasm (*arrow*). (× 8900)

lesions, the intensity of the inflammatory response is related to the degree and depth of bacterial penetration, which can be easily demonstrated by the presence of *Shigella* organisms in frozen sections, treated with fluorescein-labeled *Shigella* antiserum. By electron microscopy, intra-

epithelial *Shigella* bacilli are enclosed within membranes or lie free in the cytoplasm (Figure 3).

Comparison With Human Disease

Clinical features and gross and microscopic lesions of the colon of the monkey infected by shigellae naturally or experimentally are indistinguishable from human shigellosis.[9] Progression and appearance of mucosal lesions are identical in both hosts.

Usefulness of the Model

Given the similarity of the course and pathology of shigellosis in monkeys to human dysentery, monkey shigellosis provides an excellent model to study many facets of human *Shigella* infections. Moreover, since colonic changes in experimental monkey shigellosis are similar to other types of acute colitis in man, studies of this animal model will enhance a better understanding of acute colitis in general.

Availability

Although ecologic considerations should restrain investigators from using primates, especially wild primates, too freely, rhesus monkeys are available. As breeding colonies are expanded, they should provide enough *Shigella*-free individuals suitable for enteric studies.

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