NOTE *Laboratory Animal Science*

Colitis Associated with *Clostridium difficile* in Specific-Pathogen-Free C3H-*scid* Mice

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ABSTRACT. Soft feces and a decreased delivery rate were observed in a specific-pathogen-free (SPF) C3H-*scid* mouse breeding colony. Grossly, the ceca were shrunken and edematous in the affected mice. Histopathologically, severe edema in the cecal submucosa as well as infiltration of inflammatory cells in the lamina propria and submucosa of the ceca and colon were observed. No pathogenic microorganisms were detected by the routine microbiological tests. By anaerobic bacterial-examination, *Clostridium (C.) difficile* with toxin A was isolated from the cecal contents of the affected mice. The mice were diagnosed with *C. difficile*-associated colitis. This case appears to be the first report of natural infection with *C. difficile* in SPF mice with clinical signs.
KEY WORDS: C3H-*scid* mouse, *Clostridium difficile*, colitis.

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Clostridium difficile is known to be an agent that causes antibiotic-associated diarrhea and pseudomembranous colitis in humans [2, 5]. In laboratory animals, antibiotic-associated colitis has been reported in hamsters [1, 11, 16], guinea pigs [9, 10] and rabbits [7]. At post-administration of antibiotics such as clindamycin or penicillin, fatal enterocolitis with diarrhea, cecal dilation and hemorrhages in hamsters [11], lethal hemorrhagic cecitis with cecal dilation in guinea pigs [10] and fatal colitis with or without cecal dilation and diarrhea in hamsters [1, 16] , rabbits [7] and guinea pigs [9] have been observed. It has been reported that *C. difficile* could be isolated from germ-free rats showing diarrhea, reduction of cecal size, extensive submucosal edema and slight mononuclear cell infiltration in the cecal mucosa [4]. In contrast, conventional mice with a high isolation rate of *C. difficile* from their feces did not show pathological lesions, even after administration of antibiotics [6]. In experimental infection, germ-free mice developed diarrhea, severe decreased cecal size, and acute colitis [15]. In our SPF facility, soft feces and decreased delivery rate were clinically observed in a C3SnSmn.CB17-*Prkdc*^{scid}/J (C3H-*scid*) mouse breeding colony without any experimental treatment including administration of antibiotics. In this paper we describe the first natural case of colitis associated with the presence of *C. difficile* in SPF mice with clinical signs and suggest that *C. difficile* may be an etiological agent of colitis in SPF- immunodeficient mice.

C3H-*scid* mice were maintained as a breeding colony in our SPF facility. They were housed in sterilized aluminum cages with wood chip bedding at 24–26°C under a 12-hr light-dark cycle. Commercial food pellets (MBR-1, Funabashi Farm Co., Chiba, Japan) sterilized with gamma-rays, and chlorinated (8–13 ppm) and acidified (pH 2.5–3.0) drinking water were provided *ad libitum*. Routine bacteriological and serological examinations of retired mice, including C3H-*scid* mice, in this SPF facility were negative for *Salmonella* spp., *Citrobacter rodentium, Pasteurella pneumotropica, Pseudomonas aeruginosa, Corynebacterium kutscheri, Mycoplasma pulmonis, Clostridium piliforme,* cilia-associated respiratory bacillus, Sendai virus, mouse hepatitis virus. Endoparasites and ectoparasites were also negative. In total, 11 C3H-*scid* mice (five males and six females, age 17 days to 30 weeks) with soft feces were sacrificed by exsanguination under anaesthesia and examined microbiologically, serologically and histopathologically. Routine bacteriological and serological examinations against the above mentioned pathogens were performed. The ceca were aseptically removed and their contents as well as fresh feces were cultured anaerobically using CCFA agar (Becton Dickinson and Co., MD, U.S.A.) and GAM agar (Nissui Pharmaceutical Co., Tokyo, Japan) under the Gaspak anaerobic system (Becton Dickinson and Co., MD, U.S.A.). In selected colonies on these agars *C. difficile* were identified by BBL CRYSTAL ANR (Becton, Dickinson and Co., MD, U.S.A.) and *C. difficile*-antigen was detected by ImmunoCard *C. difficile* (Meridian Bioscience, Inc., OH, U.S.A.). Toxin A produced by *C. difficile* was detected from isolates using the Toxin Detection Kit-*C. Difficile* Toxin A Test (Oxoid Limited, Hampshire, UK; imported by Kanto Kagaku, Tokyo, Japan). For the histopathological examination, removed organs were fixed in 10% neutral buffered formalin, and prepared for light microscopy by staining with haematoxylin and eosin. Selected sections were stained by Gram's method.

Occurrence of the disease was clinically indicated by soft feces scattered on the bedding of cages holding affected mice. A decreased delivery rate (70% of normal colony, less than 50% of diseased colony) was observed in this

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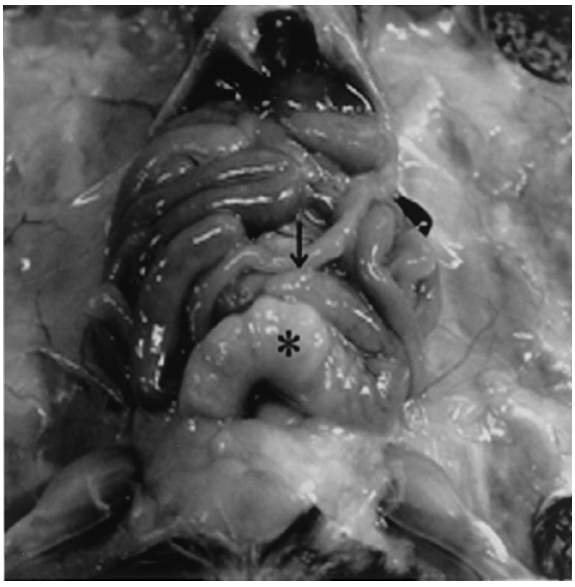

Fig. 1. The cecum from an affected C3H-*scid* mouse. The cecum is shrunken and whitish (arrow). Cecal contents seem poor. The tunica serosa coli are swollen and whitish (*).

strain. Grossly, the ceca of three mice from 11 mice were markedly shrunken and whitish, and their walls were edematous (Fig.1). Slight cecal changes were observed in the other eight mice. No gross changes were observed in the other organs. Microscopically, the lesions were limited to the ceca and colon in the 11 affected mice. The most remarkable lesions were severe edema in the submucosa with infiltration of neutrophils and mononuclear cells (Fig. 2). Infiltration of these cells was also observed in the lamina propria (Fig. 3). Many gram-positive bacilli demonstrating variable length were observed in the lumen of the ceca.

Microbiologically, routine bacteriological and serological examinations of the affected mice were negative for the above mentioned pathogens. However, gram-positive rods forming spores were isolated from the cecal contents using CCFA and GAM agars by anaerobic culturing. Isolates were identified as *C. difficile* by BBL CRYSTAL ANR. *C. difficile*-antigen was detected in the isolates by Immuno-Card *C. difficile*. Toxin A was also detectable in these isolates using the Toxin Detection Kit-*C. Difficile* Toxin A Test.

Typical lesions of the 11 affected mice were edematous colitis associated with *C. difficile*. It has been reported that colitis associated with *C. difficile* in gnotobiotic mice is pathologically characterized by decreased cecal size, polymorphonuclear cell infiltration, and edema in the lamina propria of the large intestine [15]. Vernet *et al.* have reported that inflammatory edema of the cecal submucosa occurred in mice monoassociated with moderately toxinogenic *C. difficile* [18]. Toxin A displays cytotoxic and enterotoxic activities causing an acute inflammatory response characterized by intestinal fluid secretion, epithelial damage, increased intestinal permeability, infiltration of

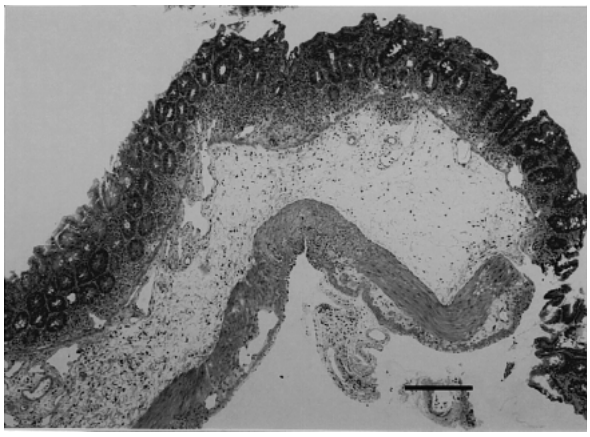

Fig. 2. The cecum from an affected C3H-*scid* mouse. Severe edema with severe infiltration of inflammatory cells can be seen in the lamina propria and submucosa. Bar=200 μm.

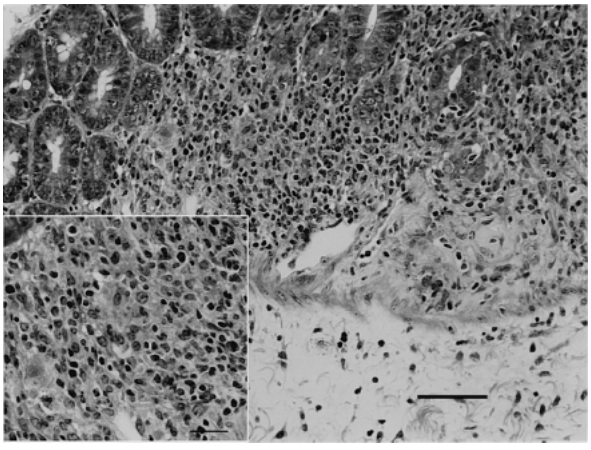

Fig. 3. Higher magnification of Fig. 2. Severe infiltration of mononuclear cells and neutrophils can be seen in the lamina propria. The intestinal crypts are dilated. Bar=50 μm. *Inset*. Higher magnification of infiltrative cells. Bar=20 μm.

the mucosa with neutrophils [12, 14, 17], and diarrhea in animals [13]. Those reports strongly suggest that our cases are *C. difficile*-associated colitis. *C. difficile* was isolated from normal mice in conventional mouse colonies, but not in SPF ones [6]. Concerning natural infection in untreated SPF animals, there is only a report on guinea pigs [3]. It has also been reported that differences in toxin receptors among animal species and disturbance of the normal intestinal flora are predisposing factors in *C. difficile*-associated disease [8]. Although the disease occurred in the C3H-*scid* mice, we examined feces from other mouse strains in the same animal room. A few *C. difficile* were isolated from some mice, which showed no clinical signs or pathological lesions. The severe immunodeficient status of C3H-*scid* mice may be responsible for the proliferation of *C. difficile* and this strain may have different toxin receptors from those of other strains. Further investigation concerning the pathogenesis of the disease in relation to immunodeficiency,

intestinal flora, and other factors is required.

In conclusion, this report appears to be the first to demonstrate naturally occurring colitis associated with *C. difficile* with clinical signs in SPF-maintained mice. If an SPF-immunodeficient mouse having digestive symptoms such as soft feces is found, examinations to reveal *C. difficile* infection will be needed.

REFERENCES

1. Bartlett, J. G., Onderdonk, A. B. and Cisneros, R. L. 1977. *Gastroenterology* **73**: 772–776.
2. Bartlett, J. G. 1997. *Semin. Gastrointest. Dis.* **8**: 12–21. (Review)
3. Boot, R., Angulo, A. F. and Walvoort, H. C. 1989. *Lab. Anim.* **23**: 203–207.
4. Czuprynski, C. J., Johnson, W. J., Balish, E. and Wilkins, T. 1983. *Infect. Immun.* **39**: 1368–1376.
5. George, R. H., Symonds, J. M., Dimock, F., Brown, J. D., Arabi, Y., Shinagawa, N., Keighley, M. R. B., Alexander-Williams, J. and Burdon, D. W. 1978. *Br. Med. J.* **1**: 695.
6. Itoh, K., Lee, W. K., Kawamura, H., Mitsuoka, T. and Magaribuchi, T. 1986. *Lab. Anim.* **20**: 266–270.
7. Katz, L., LaMont, J. T., Trier, J. S., Sonnenblick, E. B., Rothman, S. W., Broitman, S. A. and Rieth, S. 1978. *Gastroenterology* **74**: 246–252.
8. Keel, M. K. and Songer, J. G. 2006. *Vet. Pathol.* **43**: 225–240.
9. Knoop. F. C. 1979. *Infect. Immun.* **23**: 31–33.
10. Lowe, B. R., Fox, J. G. and Bartlett, J. G. 1980. *Am. J. Vet. Res.* **41**: 1277–1279.
11. Lusk, R. H., Fekety, R., Silva, J., Browne, R. A., Ringler, D. H. and Abrams, G. D. 1978. *J. Infect. Dis.* **137**: 464–475.
12. Lyerly, D. M., Lockwood, D. E., Richardson, S. H. and Wilkins, T. D. 1982. *Infect. Immun.* **35**: 1147–1150.
13. Lyerly, D. M., Saum, K. E., MacDonald, D. K. and Wilkins, T. D. 1985. *Infect. Immun.* **47**: 349–352.
14. Mitchell, T. J., Ketley, J. M., Haslam, S. C., Stephen, D. W., Burdon, D. C., Candy, A. and Daniel, R. 1986. *Gut* **27**: 78–85.
15. Onderdonk, A. B., Cisneros, R. L. and Bartlett, J. G. 1980. *Infect. Immun.* **28**: 277–282.
16. Small, J. D. 1968. *Lab. Animal Care* **18**: 411–420.
17. Triadafilopoulos, G., Pothoulakis, C., O'Brein, M. J. and LaMont, J. T. 1987. *Gastroenterology* **93**: 273–279.
18. Vernet, A., Corthier, G., Dubos, F. and Parodi, A. L. 1989. *Infect. Immun.* **57**: 2123–2127.