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PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST K99 FIMBRIAE FROM ENTEROTOXIGENIC *ESCHERICHIA COLI* AND ISOLATION OF GANGLIOSIDES RECOGNIZED BY K99 FIMBRIAE FROM BOVINE AND PORCINE SMALL INTESTINE

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Enterotoxigenic *Escherichia coli* isolated from neonatal calves, lambs, and piglets with diarrhea often possess K99 fimbrial antigen. K99 fimbriae recognize a carbohydrate epitope of glycosphingolipids at small intestinal mucosa (Ono et al., *Infect. Immun.*, 57: 907-911, 1989). Subunits mainly consisting of fimbriae are called fimbrillins, and minor adhesive subunits located at the tips of these are called adhesin. K99 adhesin has not yet been characterized.

In this communication, with the aim of isolating adhesin subunits from K99 fimbriae, a fimbriae-rich fraction was prepared by means of heat treatment (60°C, 30min), gel filtration using Sepharose 4B, and precipitation by 30% saturated ammonium sulfate. We estimated K99 activity by tracing equine erythrocyte hemagglutination (HA) activity. Ultracentrifugation at 230,000 xg concentrated the adhesin activity 60-fold, while fimbrillin subunits (18.5K) were mainly detected by SDS-PAGE.

K99 fimbriae were separated into subunits after heat treatment (100°C, 15min) in 6M urea; however, no HA or HI activity was detectable after removal of urea by dialysis.

We also tried to prepare monoclonal antibody to adhesin, which should have HA inhibitory activity. Six hybridomas were established, and only one clone (11-3.4) recognized K99 fimbrial antigen, but HA inhibitory activity was not detected. It was observed that 11-3.4 antibody recognizes a fimbrillin subunit (18.5K) by immunoblotting after SDS-PAGE and electron microscopical examination of immunostaining with colloidal gold-labeled antibody.

The content of K99 fimbrial receptor-active glycolipid which was reported to be N-glycorylneuraminyl lactosylceramide, GM3 (NeuGc) in small intestinal mucosa were compared between neonatal and adult pigs and cattle.

The amount of total gangliosides from each neonatal animal was 3 to 7 times more abundant than from each adult animal counterpart. By thin layer chromatography, it was found that both neonatal animals, especially piglets, have large amounts of GM3 (NeuGc), but that both adult pig and bovine intestines lack GM3 (NeuGc) with the exception of adult swine colon.