

SMALL INTESTINAL ANTIBODY SECRETION TO ESCHERICHIA COLI
HEAT-LABILE /LT/ ENTEROTOXIN IN PIGS

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After natural birth the newborn piglets have a large and mixed population established in the intestinal tract. These microbes are received without selection from the mother and from the immediate environment. Sow is a major source of enteric pathogens, among them of enterotoxigenic *E. coli* /EPEC/. These germs are sometimes undetectable being incorporated in the intestinal mucosal layer of the host animal. It is well established that enterotoxins play a very significant role in the pathogenesis of *E. coli* diarrheas. In the present study we presumed that ETEC strains, carried by healthy pigs, are able to produce LT enterotoxin continuously in the GI tract and pigs are capable of responding immunologically to this LT with production of LT antibodies. Data were collected of the existence and level of LT neutralizing antibodies in intestinal secretions of pigs.

Material was collected from 36 large white pigs between 20-90 days of age. Animals were fasted for 24-30 hrs. Two m long segments /mid-duodenum-mid-jejunum, D-J; mid-jejunum-mid-ileum, J-I/ were excised and washed with 20 ml saline. Intestinal contents were treated by the technique of O'Hanley and Cantley. The amount of antibodies to LT in intestinal contents was determined by the titration method of Kasai and Burrows and was expressed as anti-toxin units /AU/ per ml of intestinal contents. Secretory immunoglobulin A /IgA/ purification was done by affinity chromatography. The method involved immunoabsorbent column chromatography. Goat anti-porcine IgA was coupled to CNBr-activated Sepharose 4B/Pharmacia/ and the retained IgA was eluted with 0.05M glycine buffer at pH 2.4. Eluted porcine IgA was detected by immunoelectrophoresis /Scheidegger/ using highly purified goat anti-porcine IgA and its anti-LT activity was demonstrated in ligated rabbit ileal loops. In 20 days old animals, the LT AU/mg protein values /specific activity/ were relatively high. High level of total LT AU was also found in both segments. The 50 days old pigs, after 12-16 days of their weaning, showed lower titers of LT neutralizing activity: about 1/3 to 1/2 of the 20 days old piglets' values. At 75 and 90 days of age a raise in titers was observed in the specific activity and in total LT AU contents as well: values reached or exceeded the 20 days old animals' titers. Analysing these data, a rising tendency of anti-LT titers increasing by age could be observed. Control rabbit intestinal contents were free from anti-LT titers. The synthesis of anti-LT antibodies increased when pigs were fed with heat-treated LT containing *E. coli* antigens or vaccinated im. with LT containing complex vaccine, and markedly decreased when animals, diseased in *E. coli* diarrhea, were examined. Immunoelectrophoretic analysis of secretions from pigs of different age showed that with specific goat anti-porcine IgA reagent IgA was readily detectable in every tested intestinal secretions and immunoabsorbent-purified IgA neutralized LT toxin activity in rabbit ileal loops.

Exposure to ETEC strains is fairly widespread in our country and occurs early in life. These strains are enterotoxin producers and LT toxin is a good antigen being a protein in nature. Pigs are responsive immunologically to LT toxin which is continuously

produced by nonindigenous *E. coli* strains carried in the intestinal tract of healthy pigs. LT toxin plays a significant role in the pathomechanism of *E. coli* diarrheas. Well-detectable amounts of LT antibodies were present in D-J and in J-I contents and gradual rise in LT antitoxin titers was observed with progressing age. The high anti-LT values in 20 days old pigs can be related, partly at least, to the milk of the sow. These animals have suckled their dams before extermination. The specificity of the anti-LT activity in the small intestinal secretions is suggested by the following. /1/ Antibodies capable of passively neutralizing LT toxin preparations were demonstrated in the intestinal secretions of pigs. /2/ After withdrawal of the dams' milk, titers decreased. /3/ *E. coli* LT antigenic stimulus increased the secretion of LT neutralizing antibodies. /4/ In *E. coli* diarrhea cases LT antibody levels in intestinal secretions decreased significantly. /5/ LT antibody activity in secretions was predominantly associated with IgA. /6/ LT neutralizing antibodies were demonstrated not only in intestinal secretions but also in sera of pigs belonging to comparable age groups. /7/ Control rabbits did not exhibit any anti-LT activity in their intestinal contents. /8/ Heating the secretions at 56°C /30 min/ before testing failed to produce any change in anti-LT titers.

LT antibody activity in intestinal secretions confers presumably to the so-called natural resistance against artificial *E. coli* infection usually observed in pigs older than 10-20 days of age. Under normal feeding and environmental conditions the high amount of LT antibodies, associated with IgA and forming a mucin bound barrier on the intestinal mucosa provides significant protection against the development of *E. coli* diarrhea. Up to now, data are not available about specific anti-LT activity in pig small intestinal secretion tested with purified LT toxin and purified IgA. Our studies provide satisfactory evidence that anti-LT antibody appeared in the lumen of small intestines of pigs. The lower level of anti-LT activity found in secretions of 50 days old pigs suggests that in this period pigs have a critical LT antibody deficiency which adversely affects the intestinal defense mechanism of the animals. The phenomenon can be related to the occurrence of the frequently observed post-weaning *E. coli* diarrhea. In the immediate pre-weaning and post-weaning period administration of LT containing antigen by either the parenteral or oral route induced higher level of mucosal immunity. The nature of the "paralysis" of the well-defined defense mechanism in naturally occurring *E. coli* disease is still not known. Summary. With purified LT toxin and IgA specific anti-LT activity was demonstrated in small intestinal contents of pigs. Gradual rise of intestinal LT antitoxin titer was observed with progressing age. Feeding or injecting LT containing antigen increased LT antibody level in the secretions. In *E. coli* diarrhea LT antibody levels in intestinal secretions decreased significantly. Kasai G.J. and W. Burrows: J. Infect. Dis., 1966, 116, 606-614. O'Hanley, P.D. and J.R. Cantley: Infect. Immun. 1981, 31, 316-322.