

ELECTRON MICROSCOPY OF THE COLONIC EPITHELIUM OF PIGS INFECTED

WITH SPIROCHETES ASSOCIATED WITH SWINE DYSENTERY
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Spirochetes are regularly isolated from the feces of pigs with swine dysentery and have also been demonstrated in the colon of diseased pigs. In spite of extensive research, very little is known about the mechanisms by which the colitis is initiated. The present knowledge is mainly based on results obtained by light microscopy. Here we report on a study of the ultrastructure of the colonic epithelium in biopsies taken from pigs fed spirochetes isolated from pigs with and without manifest swine dysentery.

Eight specific pathogen free pigs, seven weeks old, were used for the experiment. The pigs were divided in four groups of two. While one group was kept as controls, the rest of the pigs were, on two consecutive days, each fed 250 ml homogenate of blood agar plates with confluent growth of three different strains of spirochetes. These strains had been isolated during routine diagnostic work. One, a weakly hemolytic strain (Dys 112) was isolated from a herd with diarrhoea problems. Another (Dys 197) was isolated from a herd where diarrhoea was a frequent problem. This strain showed an intermediate hemolytic activity. The third strain (Dys 118) was isolated from an outbreak of swine dysentery and was strongly hemolytic. The strains were tested by the API ZYM system and by agglutination with immune sera against different serotypes of *T. hyodysenteriae* and *T. innocens*.

The pigs were regularly tested for the presence of spirochetes, by rectal swabs. The pigs fed Dys 112 were positive on days 7, 11, and 18, while the pigs fed Dys 197 and Dys 118 were all negative until day 18 after their first exposure to the spirochetes. None of the pigs showed any clinical symptoms.

All eight pigs were killed and bled on day 25 and immediately necropsied. No alterations of the colonic mucosa were macroscopically visible at necropsy.

Small segments of the colon from one pig of each group were excised, ligated at both ends, and injected with 6% glutaraldehyde in cacodylate buffer before the whole segment was immersed in the same fixative. After fixation for about an hour, parts of the colon segments were cut in small pieces. These were further fixed in the same fixative for a total of 4 hours. The biopsies were stored in 0.2 M sucrose in cacodylate buffer at +4°C until further processed by postfixation in 1% OsO₄, en bloc treatment with uranyl acetate, dehydration in alcohol, and embedding in Vestopal W.

One to two micron thick sections were stained with toluidine blue for light microscopy and ultrathin sections were stained with lead and uranyl salts.

The ultrastructure of the colonic epithelial cells was similar for the control pig and the pig fed Dys 112, i.e., the weakly hemolytic spirochete. The luminal epithelial cells possessed numerous microvilli, whereas microvilli were more sparse on cells in the crypts. The cell membrane of the epithelial cells were tightly interdigitated and numerous desmosomes were present. Tight junctions were seen between the cells close to the intestinal lumen. Mitochondria with well preserved cristae, well developed Golgi zones, and tightly apposed strands of rough endoplasmic reticulum as well as a great number of free ribosomes, were seen in the cell cytoplasm. Membrane bound electron dense inclusions of unknown origin were occasionally seen close to the lumen in both the columnar and the goblet cells. Luminal cells which were not well preserved were observed now and then. These were probably cells that were about to slough off. Invasive cells were observed a few times. Both gram-positive and gram-negative bacteria were found in the lumen and in the crypts.

The ultrastructure of the epithelium was also similar for the pigs fed strains Dys 197 and Dys 118, which showed intermediate and strong hemolytic activity, respectively.

Generally the columnar cells in the biopsies of

these pigs were shorter and more cuboidal than in those of the previous pigs. The microvilli were few and short also on the luminal cells, and very little glycocalyx was present on and around the microvilli. Inter-cellular gaps were present rather frequently between the epithelial cells. In the cytoplasm the mitochondria appeared swollen or bursted, and the rough endoplasmic reticulum appeared somewhat dilated. Well preserved cells also occurred in these biopsies. Membrane bound electron dense inclusions were seen, and were especially prominent in goblet cells in the crypts of the pig inoculated with Dys 118. Goblet cells constituted a very high proportion of the epithelial cells in the crypts. Leukocytes were occasionally observed between cells.

A few spirochetes were found at the orifices of some of the crypts. Gram-positive and gram-negative bacteria occurred in the lumen as well as in the crypts. It appeared that the bacteria were more numerous and showed greater morphologic differences in these pigs than in the control pig and the pig fed the weakly hemolytic strain of spirochetes. However, the material is as yet too limited for an evaluation of the frequency with which different types of bacteria occurred.

It is interesting that it seems possible on basis of the ultrastructure of the colonic epithelium to divide the clinically healthy pigs into two groups, which can be related to the hemolytic activity of the spirochetes with which they were fed. The alterations observed in the cell ultrastructure resemble a toxic reaction. Evidence of the existence of a toxin has hitherto been sought in vain by ordinary histological techniques. Further studies are obviously needed before we fully understand the pathogenesis of swine dysentery and the implications it may have that clinically normal pigs can harbour strongly hemolytic spirochetes.

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