SURFACE COMPONENTS OF BORDETELLA BRONCHISEPTICA AND THEIR RELATIONSHIP TO SEROLOGICAL REACTIVITY WITH PIG, RABBIT AND MOUSE ANTIBODIES.

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Fresh isolates of B.brochiseptica obtained from pigs with atrophic rhinitis were found to exhibit a complex surface structure. Although flagella were relatively rare, all cells contained a few fimbriae and a fine fibrillar meshwork which covered the surface of cells and which was, morphologically, not dissimilar to the K88 antigen of E.coli. When viewed on transparent solid media using transmitted light, most fresh isolates appeared as "light coloured" colonial forms (L). However, in one strain "dark coloured" colonies (D) were observed - cells from these colonies appear to differ in the proportion of their surface appendages; D-variants evidently contain more fimbriae and less fibrillar meshwork than the L-variants, and they also differ in their outer membrane protein composition. Both of these colonial forms are smooth ("Phase I"), both are similarly virulent for mice, both protect mice against intraperitoneal challenge and both agglutinate with K2, K3 and K4 sera (Pedersen, 1975).

Attempts were made to determine the virulence of fresh isolates, and of the L and D colonial forms in particular, using strips of embedded rabbit (or pig) tracheal cultures (Matsuyama, 1974). However, after point inoculation with 2 - 4 CFU of all the B.brochiseptica strains tested, multiplication in the tracheal culture was very fast; all of the strains showed resistance to the "cleansing effect" of the tracheal mucosa. Using this method, as in the experiments of Bemis and Kennedy (1981) with isolated ciliated epithelial cells, differences in virulence between strains are not apparent. However, when we sectioned the tracheal cultures 14-16 hours after point inoculation, we found that some strains (including both L and D colonial forms) had a tendency to "penetrate" deep into the mucosal layer, and B.brochiseptica cells could be found between bundles of cartilages. Unfortunately, we did not have the chance to investigate this ability to penetrate the deep layers of mucosa using B.brochiseptica isolates obtained from pigs not showing clinical atrophic rhinitis.

A "K-antigen" preparation (Ørskov, Ørskov, Jann and Jann, 1977) of B.brochiseptica, which consists mainly of the fibrillar antigen and outer membrane proteins, was used in ELISA to determine the immune status of mice previously injected with graded doses of B.brochiseptica vaccines. By determining the level of antibodies reacting with this antigenic mixture, it was possible to predict whether or not a mouse would survive an intraperitoneal B.brochiseptica challenge. Similarly, ELISA was used to detect the level of antibodies in sera and colostra of vaccinated sows, of passively transferred antibodies in sera of piglets and of actively produced antibodies in infected piglets. However, after the fractionation of the "K-antigen" preparation and the analysis of the titres of antibodies reacting with its individual components, it appears that only those antibodies which reacted with the outer membrane protein moiety related to the level of protection against atrophic rhinitis; antibodies which reacted with the fibrillar antigen and/or the lipopolysaccharide (present as a minor contamination of the preparation) had no such relationship.

When the outer membrane protein complex was subjected to SDS-PAGE, followed by electroblotting to nitrocellulose (Towbin, Staehelin and Gordon, 1979), it was found that the most serologically reactive portion was a complex which consisted of a 44 KD heat-modifiable protein, 39 and 33 KD proteins, and a protein showing an apparent molecular weight of approximatley 54 KD. This latter antigen is, however, exceptional; it loses its reactivity against antisera when heated for 5 minutes at 100°C with 1% sodium dodecylsulphate in the presence of 0.05 M dithiothreitol, but retains its high serological reactivity when heated in the same solution for 1 minute at only 56°C. It is, however, not clear whether these antigens, when isolated, will prove to be the protective B.brochiseptica antigens.

Conclusions: The level of pig serum antibodies reacting with the outer membrane proteins of B.brochiseptica appear to reflect resistance to atrophic rhinitis caused by this microorganism.

Selected references: Pedersen, K.B.; Acta path.microbiol.scand. Sect.B. 1975, 83:590. Matsuyama, T.; J.infect.Dis. 1974, 130:508. Bemis, D.A. and Kennedy, J.R.; J.infect.Dis. 1981, 144:349. Ørskov, I., Ørskov, F., Jann, B. and Jann, K.; Bact.Reviews, 1977, 41:667. Towbin, H., Staehelin, T. and Gordon, J.; Proc.Natl.Acad.Sci. 1979, 76:4350.