

How far are we away from oral dead vaccines? Insights from oral vaccinations with F4 and F18 fimbriae from *E. coli* in the pig

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Vaccination against intestinal infections remains a tremendous challenge. Both parenteral vaccines, which tend to stimulate the systemic rather than the mucosal immune system, and oral vaccines, containing non-replicating antigens, have generally been ineffective in protecting against mucosal pathogens. On the other hand, oral administration of live organisms can be effective, but is often accompanied with clinical signs and if not, generally confers only partial protection against infection. One of the reasons that very few antigens can induce an intestinal immune response when given orally is that the dominant response mode of the mucosal immune system is immunological tolerance. The best-known examples of soluble oral immunogens are cholera toxin and the heat-labile enterotoxin (LT) of *E. coli*². We have proven that in pigs the purified F4 fimbriae of enterotoxigenic *E. coli* (ETEC) can be added to this list of rare soluble oral immunogens, since oral administration of these fimbriae to F4 receptor positive (F4R⁺) pigs actively induces an intestinal immune response⁸ with induction of F4-specific IgA, providing complete protection against challenge with F4⁺ ETEC⁹. Interestingly most if not all of the soluble antigens that are immunogenic via the oral route bind to receptors on enterocytes. For instance the B subunit of cholera toxin binds to ganglioside receptors and wheat germ agglutinin (WGA) binds to the epidermal growth factor receptor. A unique feature of the F4 model is that there are pigs with and without receptors for F4 fimbriae on their small intestinal villous enterocytes. This allowed us to demonstrate that only pigs with the F4R show an intestinal immune response⁸. In pigs without the F4R, F4 behaves as a food antigen¹¹. Most likely uptake in and transcytose through enterocytes is one of the mechanism via which oral antigen reaches the underlying gut-associated lymphoid tissue⁵. For F4, we identified aminopeptidase N as a receptor⁴ involved in endocytosis. Transcytosis of F4 by enterocytes was demonstrated *in vitro* using an intestinal epithelial cell line (IPEC-J2)⁴ and *in vivo* using intestinal loops⁷. Uptake by enterocytes can lead to an intestinal immune response⁶. However, *in vivo* also binding to and uptake by M cells could be seen⁷ and injection of F4 at different sites in the intestinal tract showed that jejunal Peyer's patches are the major inductive site of the F4-specific mucosal immune response⁶. After uptake by the epithelial cells, F4 could be seen in CD172a⁺ cells. This molecule is expressed by dendritic cells (DCs). Activation and maturation of DCs plays a crucial role in induction of a mucosal immune response. When we incubated DCs with F4, this induced their maturation³. Another feature of immunogenic soluble oral antigens is that they are often polymeric in nature: cholera toxin B is a pentamer, WGA a dimer and F4 a polymer of 100 to 1000 subunits⁹. We demonstrated that this polymeric nature of F4 is important for its mucosal immunogenicity¹⁴.

The crucial role of the receptor in the mucosal immunogenicity was also demonstrated by immunizing pigs with the purified fimbriae of F18+ verotoxigenic. These fimbriae adhere to blood group ABH type 1 determinants on glycosphingolipids in the brush border of enterocytes¹. Binding to this receptor does not result in endocytosis and no immune response is induced by oral immunizing pigs with amounts 30 times higher than the dose used for the F4 immunization¹³.

In our lab, the oral F4 model is not only used to study intestinal immune mechanisms in the pig, but also to evaluate the effect of potential adjuvants such as β glucans and to target non-immunogenic antigens to the gut-associated lymphoid tissue¹².

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