Development and Evaluation of Vaccines based on Fimbrial Adhesins of *Escherichia coli*

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Abstract

With most pathogens acquiring resistance to currently used drugs, the development and formulation of vaccines against predominant infectious diseases has taken centre stage. Bacterial pathogens utilize a wide variety of virulence factors that are critical for disease, and therefore make attractive vaccine candidates. Fimbriae are one type of these virulence factors and mediate adherence to host tissues. Since preventing attachment of a bacterial pathogen is often adequate to render it non-virulent, anti-adhesin antibodies can play an important role in the protection against infection.

The aim of this Ph.D. thesis therefore concerned the design, construction and evaluation of recombinant anti-adhesin vaccines. The major research focus lay on the use of live-attenuated *Salmonella* vaccine strains as vectors to express and deliver fimbrial receptor-binding domains.

We have chosen to focus our research on two fimbriae: type 1 and F17a fimbriae. Both fimbriae play a role in the initial stages of infection and sufficient structural information is present to identify their receptor-binding domains. Type 1 fimbriae, although present on most *Enterobacteriaceae*, are specific virulence factors of avian pathogenic *E. coli* (APEC) and uropathogenic *E. coli* (UPEC). The adhesin of type 1 fimbriae, FimH, allows binding to epithelial cells of the respiratory tract of poultry and the bladder of mammals. F17a fimbriae are found on certain bovine enterotoxigenic *E. coli* (ETEC) strains that inflict severe diarrhea in new-borne calves, and their adhesin, F17a-G, mediates attachment to the intestinal epithelium of calves. As the FimH and F17a-G adhesins are unstable in the absence of their chaperones, due to the incomplete Ig fold of the pillin domain, we used constructs covering only the receptor-binding

domains of the adhesins. These domains do have a complete Ig fold and are therefore stable entities on their own.

Since purifying large quantities of adhesins is not cost-effective, the engineering of an antigen delivery system was contemplated. Using live-attenuated *Salmonella* strains as antigen delivery systems has the additional advantage that immune responses along the mucosa can be induced. Given that surface expression was known to enhance the immune response to heterologous antigens, the receptor-binding domains of the FimH and F17a-G adhesins were targeted to the surface of the bacteria by fusing them to the translocator domain of the autotransporter protein AIDA-I. Since in the literature the expression of different antigens driven by the *in vivo* inducible P_{pagC} promoter was able to elicit strong immune responses in mice, this promoter was chosen to drive the production of the adhesin-autotransporter fusion proteins.

Initial tests, such as outer-membrane preparations, whole cell ELISA using an anti-adhesin antibody and surface protease accessibility, indicated that the receptor-binding domains of both adhesins seemed accessible at the bacterial surface. An IgG response against LPS, but not against the receptor binding domains, was induced after immunization of mice with a live-attenuated *S. enterica* serovar Typhimurium strain displaying the fimbrial receptor-binding domains.

Since surface-exposure of antigens can be critical for eliciting an antibody response, we further investigated the location of the F17a-G receptor-binding domain in bacterial cells. Induction of the synthesis of the fusion protein seemed however to permeabilize the cells. Possibly an excessive expression level, combined with an incomplete translocation of the F17a-G passenger domain, resulted in an altered integrity of the outer membrane. This can conceivably result in a detrimental additional attenuation, reducing the efficacy of the vaccine strain.

In an attempt to optimize the *in vivo* expression level of the receptor-binding domain in *Salmonella*, other promoters than P_{pagC} were tested. The search for suitable promoters is however hampered by the fact that i*n vitro* mimicking of the stimuli controlling *in vivo*-inducible bacterial promoters during infection of the host can be complex. Therefore the use of the nematode *Caenorhabditis elegans* was evaluated, as a surrogate host to examine the expression of *Salmonella enterica* promoters. Green fluorescent protein (GFP+) was put under the control of the promoters of the *pagC, mgtB, sseA, pgtE* and *fur* genes of *S. enterica*. After infection of *C. elegans* with a *S.* Typhimurium vaccine strain expressing these constructs, clear bacterial expression

of GFP+ was observed under the control of all five promoters, although significant expression was not always obtained *in vitro*. It was therefore concluded that *C. elegans* constitutes a valuable model system for the study of the *in vivo* expression of *Salmonella* promoters.

Tsh, a temperature-sensitive hemagglutinin for chicken erythrocytes, is a potential virulence factor of APEC, and was employed for testing the use of the different promoters. After immunization of mice with a *S.* Typhimurium vaccine strain expressing the *tsh* gene under the control of the promoters of the *pagC*, *mgtB*, *sseA*, *pgtE* and *fur* genes, no measurable antibody titers against Tsh were detected in sera, although a response against LPS was seen.

In a second strategy, inactivated bacteria were tested. After immunization of mice with acetone-inactivated *Salmonella* expressing the adhesin-AIDA-I fusion proteins, an efficient IgG response against F17a-G or FimH was induced. Fusion to the AIDA-I translocator domain was further found to be beneficial for antibody production, compared to periplasmic expression of the free F17a-G receptor-binding domain or to immunization with bacteria carrying complete F17a fimbriae. Additionally, the use of the *Salmonella* strain as carrier was superior to using the *E. coli* strain.

Surprisingly, mice vaccinated with acetone-inactivated *Salmonella* with and without the FimH-AIDA-I fusion protein were both protected against UTI challenge, although only sera of mice vaccinated with *Salmonella* expressing the fusion protein contained detectable serum IgG titers against FimH. Not the presence of a specific immune reaction against FimH, but rather a general humoral or cellular activation of the immune system after immunization may account for the observed protection.

The principal focus of the experimental investigations in the previous chapters was to develop an *in vivo* display system for F17a-G using the autotransporter AIDA-I. However, seeing that the use of AIDA-I was not successful for this aim, we investigated the use of filamentous phage display as immunization method for F17a-G. The receptor-binding domain of the adhesin was expressed on bacteriophage M13, as an N-terminal fusion with the phage protein pIII. The domain retained its sugar binding activity. Phage presenting the fimbrial receptor-binding domain elicited an IgG response against F17a-G after intraperitoneal immunization of mice, but titers were not sufficient to inhibit binding of F17a-G to GlcNAc-containing receptors. Fusion of F17a-G to pVIII was also carried out, but most likely the incorporation of the fusion proteins disrupted phage assembly. Preliminary tests to employ a live-

attenuated *Salmonella* vaccine strain infected with M13 for the continuous delivery of the phages to the immune system were conducted. After nasal immunization with this strain infected with M13, mice showed high IgG titers against the phage. Successful introduction in *Salmonella* of the F-plasmid necessary for M13 infection and the Gateway plasmid containing the fusion protein under the control of the *in vivo* inducible P_{pagC} promoter was however not yet achieved. Immunization with M13 infected gut-colonizing *E. coli,* isolated from murine feces, did on the other hand not yield any anti-phage antibodies.

To complement the results obtained for phage display of F17a-G, experiments were repeated using the FimH receptor-binding domain. The results are consistent with the ones presented for F17a-G and demonstrated that the receptor-binding domain of FimH could also be functionally displayed by filamentous bacteriophages, with preservation of its sugar-binding specificity. Again, after immunization with phages displaying the FimH domain, a humoral IgG response against FimH was elicited in vaccinated mice.