Vaccination against cholera and ETEC diarrhea and interventions to improve vaccine immune responses

Akademisk avhandling

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av

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Avhandlingen baseras på följande arbeten:

I Qadri F, Ahmed T, Ahmed F, Begum YA, Sack DA and Svennerholm AM: Reduced doses of oral killed enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine is safe and immunogenic in Bangladeshi infants 6–17 months of age: Dosing studies in different age groups.
Vaccine 24, 1726-33, 2006.

II Qadri F, Ahmed T, Ahmed F, Bhuiyan MS, Mostofa MG, Cassels FJ, Helander A and Svennerholm AM: Mucosal and systemic immune responses in patients with diarrhea due to CS6-expressing enterotoxigenic *Escherichia coli*.
Infect Immun 75, 2269-74, 2007.

III Ahmed T, Lundgren A, Arifuzzaman M, Qadri F, Teneberg S, Svennerholm AM:
Children with Lewis (a+b-) blood group have increased susceptibility to diarrhea caused by enterotoxigenic *Escherichia coli* expressing colonization factor I-group fimbriae.
Infect Immun 77, 2059-2064, 2009.

IV Ahmed T, Svennerholm AM, Tarique AA, Sultana GN and Qadri F: Enhanced immunogenicity of an oral inactivated cholera vaccine in infants in Bangladesh obtained by zinc supplementation and by temporary withholding breast feeding.
Vaccine 27, 1433-1439, 2009.

V Ahmed T, Arifuzzaman M, Lebens M, Qadri F, Lundgren A: CD4+ T-cell responses to an oral inactivated cholera vaccine in young children in a cholera endemic country and the enhancing effect of zinc supplementation.
Submitted for publication.

GÖTEBORGS UNIVERSITET

Vaccination against cholera and ETEC diarrhea and interventions to improve vaccine immune responses

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Abstract
Vibrio cholerae O1 and enterotoxigenic *Escherichia coli* (ETEC) together account for the majority of bacterial causes of acute dehydrating diarrhea in children in Bangladesh. Vaccines should be considered as an important public health tool for prevention of these diarrheal diseases. However, a limitation for the use of vaccines in developing countries is that the efficacy and immunogenicity of vaccines, especially oral enteric vaccines, are lower in these countries than in the industrialized world. The main objectives of the thesis were to study the safety and immunogenicity of oral cholera toxin B subunit (CTB) containing inactivated whole cell ETEC and cholera vaccines in young children in a developing country and to identify possible immune modulating factors, e.g. vaccine dose, different buffer formulations, effects of breast milk withholding and zinc supplementation.

For determining optimal doses of the ETEC vaccine, we immunized 6 months to 12 year old children with full, half and quarter doses of the ETEC vaccine. Safety and immunogenicity of different vaccine doses were compared. All doses of the ETEC vaccine were found to be equally immunogenic in the older children. However, a quarter dose, although giving somewhat lower antibacterial responses than a full dose, was required for children 6-18 months to avoid reactogenicity.

For determining the safety and immunogenicity of the cholera vaccine in young children and the effect of different interventions to try to enhance immune responses, children 6-18 months of age were given two doses of the vaccine according to the standard protocol or with different modifications. In addition to analyzing antibacterial and antitoxic B-cell responses, T-cell responses were determined using a new flowcytometric technique, FASCIA. The vaccine was found to be safe and to induce both antibody and Th1 type T-cell responses. Vibriocidal antibody responses were improved by temporarily withholding breast-feeding for three hours before immunization as well as by giving 20 mg of zinc from 3 weeks prior to and one week after the second dose of vaccine. Zinc supplementation also enhanced IFN-γ responses to CTB.

Further objectives of this thesis were to analyze the immune responses to one of the most prevalent ETEC colonization factors (CFs), i.e. CS6, in patients infected with CS6-positive ETEC and to evaluate if there is an association between expression of certain Lewis blood group antigens of the host and infection by ETEC expressing different CFs. Natural infection with CS6 ETEC was found to induce robust systemic and mucosal immune responses in 70-90% of adults and children with diarrhea caused by CS6 positive ETEC strains, suggesting that CS6 could be an important immunogenic component of a new ETEC vaccine. We could also show that individuals with Le (a+b-) blood group had increased susceptibility to infection with ETEC expressing CFA/I group fimbriae.

The results of these studies give important background information regarding the possibility of inducing effective immune responses to oral inactivated enteric vaccines in young children in developing countries.

Keywords: *Vibrio cholerae*, ETEC, oral vaccine, CS6, CFA/I, Lewis blood group, zinc, breast feeding, T cell, B cell

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