

A mouse model for direct evaluation of cholera vaccines

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien vid Göteborgs universitet kommer att offentligen försvaras i hörsal Ivan Östholm, Medicinargatan 13, Göteborg, torsdagen den 7 Maj 2009 kl. 13.00

av

Erik Nygren

Fakultetsopponent:

Björn Gustafsson, Docent i mikrobiologi vid Karolinska Institutet samt forsknings och utvecklingschef vid Crucell SBL vaccines, Stockholm

Avhandlingen baseras på följande arbeten:

- I. Nygren E, Holmgren J, Attridge SR
Murine antibody responses following systemic or mucosal immunization with viable or inactivated *Vibrio cholerae*
Vaccine. 2008 Dec 9;26(52):6784-90.
- II. Nygren E, Li Bl, Holmgren J, Attridge SR
Establishment of an adult mouse model for direct evaluation of the efficacy of vaccines against *Vibrio cholerae*
Submitted
- III. Nygren E, Holmgren J, Attridge SR
Immunogenicity of live and killed *Vibrio cholerae* O1 and O139 oral vaccines in an adult mouse model: cholera toxin adjuvants intestinal antibody responses and serogroup homologous and cross-reactive protection
Submitted



GÖTEBORGS UNIVERSITET

A mouse model for direct evaluation of cholera vaccines

Erik Nygren

Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Sweden, 2009

Abstract

Cholera continues to be an important cause of morbidity and mortality in large parts of the developing world and is a significant negative factor for economic development. *Vibrio cholerae* bacteria of the O1 or O139 serogroup can cause disease due to their ability to colonize the intestine and produce an enterotoxin, cholera toxin (CT). An effective oral vaccine against *V. cholerae* O1 is available, whereas vaccine against O139 is lacking. Development and pre-clinical evaluation of cholera vaccines have been hampered by the fact that man is the only natural host for *V. cholerae*. Although various animal models have been described, there exists no convenient and inexpensive model that allows evaluation of vaccine-induced protection against a challenge infection.

The main objective of this thesis was to develop a model that allows direct evaluation of the immunogenicity and protective efficacy of cholera vaccine candidates in conventional adult mice. Paper I demonstrates that strong serum and mucosal antibody responses to *V. cholerae* O1 or O139 lipopolysaccharide (LPS) can be induced in adult mice vaccinated intranasally or orally with either live or formalin-killed bacteria. Standardized intestinal IgA antibody responses estimated using extracts prepared from faecal pellets or from intestinal mucosa were found to correlate significantly, hence validating the use of the more convenient fecal pellets extracts for measuring gut mucosal antibody responses in vaccinated hosts. Paper II describes an adult mouse model for studying intestinal colonization by *V. cholerae* and associated immune responses. It was shown that oral pre-treatment of mice with streptomycin (Sm) allows intestinal colonization by Sm-resistant *V. cholerae* O1 or O139 bacteria, and that mice immunized with viable or inactivated *V. cholerae* as described in Paper I were comparatively refractory to colonization following infection/challenge with the immunizing strain, with protection resulting in accelerated clearance of the challenge organisms correlating inversely with the intestinal IgA anti-LPS response. In paper III this model was further used to evaluate immune responses and protection by orally administered live and killed O1 and O139 whole cell vaccines and the impact of co-administration of CT on the immunogenicity and protective effect. CT proved to be an effective adjuvant, markedly potentiating antibody responses and also increasing the protective effect against both serogroup homologous and heterologous challenge. The results presented in this thesis suggest that the new adult mouse model may be used to broaden our understanding of immune protection against *V. cholerae* infection, and thus be a useful tool in the pre-clinical evaluation of oral cholera vaccines.

Keywords: *Vibrio cholerae*, cholera vaccine, cholera toxin, LPS, anti-bacterial immunity, IgA, challenge, protection, colonization

ISBN 978-91-628-7777-4