Approaches to Enhance and Evaluate the Immunogenicity of an Oral ETEC Vaccine

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal
Ivan Östholm, Medicinaregatan 13, Göteborg

Torsdagen den 26:e november 2015, kl. 0900

av

Susannah Leach

Fakultetsoppponent
Professor Harleen Grewal
Institutet för Klinisk Vetenskap
Universitetet i Bergen, Norge

Avhandlingen baseras på följande arbeten:

I. Clinical trial to evaluate safety and immunogenicity of an oral inactivated enterotoxigenic Escherichia coli prototype vaccine containing CFA/I overexpressing bacteria and recombinantly produced LTB/CTB hybrid protein.

II. Different kinetics of circulating antibody-secreting cell responses after primary and booster oral immunizations: a tool for assessing immunological memory.

III. The adjuvant double mutant Escherichia coli heat labile toxin enhances IL-17A production in human T cells specific for bacterial vaccine antigens.

IV. Cross-reactivity and avidity of antibody responses induced by an oral, multivalent enterotoxigenic Escherichia coli (ETEC) vaccine.
    Leach S, Lundgren A, Carlin N, Lofstrand M, Svennerholm AM. In manuscript.
Approaches to Enhance and Evaluate the Immunogenicity of an Oral ETEC Vaccine

Susannah Leach, Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Sweden

Enterotoxigenic Escherichia coli (ETEC) is a major cause of childhood diarrhoea in the developing world and the most common cause of travellers’ diarrhoea. A new oral multivalent ETEC vaccine (MEV) containing killed recombinant E. coli bacteria expressing increased levels of the most prevalent ETEC colonisation factors (CFs), i.e. CFA/I, CS3, CS5 and CS6, and the toxoid LCTBA, a hybrid between the binding subunits of ETEC heat labile toxin (LTB) and cholera toxin (CTB), has been developed at the University of Gothenburg. The main aim of this thesis was to analyse immune responses induced by MEV and related vaccines in humans, and to evaluate different approaches to enhance and measure such responses.

The safety and immunogenicity of two oral doses of a prototype of MEV, containing CFA/I over-expressing E. coli bacteria and LCTBA, were evaluated in a Phase I trial in adult Swedish volunteers. The vaccine was safe and induced significant faecal secretory IgA and intestine-derived antibody-secreting cell (ASC) IgA responses in peripheral blood against CFA/I and LTB, as well as IL-17A and IFNγ T cell responses to LTB. However, detailed studies of the kinetics of ASC responses induced by an oral inactivated model vaccine, the CTB-containing cholera vaccine Dukoral®, indicated that peak ASC responses may have been missed in the prototype ETEC vaccine trial assessing ASC responses 7 days after each vaccine dose. Thus, whereas CTB-specific ASC responses to Dukoral® peaked around 9 days after the first dose, ASC responses to a second or late booster dose (given 6 months - 14 years later) peaked already on day 4-5. The distinct kinetics of ASC responses to primary and booster vaccinations suggests that early peak ASC responses may indicate the presence of mucosal B cell memory.

In preparation for testing MEV with the mucosal adjuvant double-mutant LT (dmLT), we evaluated the effect of dmLT on human T cell responses in vitro. dmLT enhanced both IL-17A and IFNγ responses to LTB in cells from ETEC vaccinees and IL-17A responses to mycobacterial antigens in cells from BCG vaccinees; this effect was dependent on IL-1β and IL-23, and could be mediated via monocytes.

We also studied the functional characteristics of the antibody responses induced by MEV. Two oral doses administered ± dmLT to adult Swedish volunteers, as well as a single booster dose administered 13-23 months later, induced cross-reactive mucosal antibody responses to multiple related, non-vaccine CFs. Using a novel assay, we showed that the avidity of both mucosal and serum antibodies to key vaccine antigens increased in response to the late booster dose.

Collectively, our results indicate that MEV can induce mucosal antibodies with the potential to protect against a broad range of ETEC strains. Our demonstration that dmLT can enhance T cell responses indicates that dmLT may promote B cell differentiation and memory development. Our studies of the kinetics of ASC responses have indicated optimal sampling time points for performing such analyses and established a method for memory assessment. These results are important for continued clinical evaluation of the new ETEC vaccine.

Key words: ETEC, vaccine, adjuvant, human, mucosa, antibody, cross-reactivity, avidity, T cell, immunological memory