Göteborg, 2019

Novel approaches to mucosal vaccine development

Strategies in vaccine antigen production, construction of a novel mucosal adjuvant and studies of its mode of action

AKADEMISK AVHANDLING

Som för avläggande av medicin doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal 1034, Ivan Ivarsson, Medicinaregatan 3A, Göteborg, Fredagen den 6 December 2019 , klockan 13.00

Av **Manuela Terrinoni**

Fakultetsopponent:
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Avhandlingen baseras på följande delarbeten

I. Manuela Terrinoni, Stefan L. Nordqvist, Susanne Källgård, Jan Holmgren, Michael Lebens
A novel non antibiotic, lgt-Based selection system for stable maintenance of expression vectors in Escherichia coli and Vibrio cholerae.
Applied Environmental Microbiology 2017 84:e02143-17.
https://doi.org/10.1128/AEM.02143-17.

II. Michael Lebens, Manuela Terrinoni, Stefan L. Karlsson, Maximilian Larena, Tobias Gustafsson-Hedberg, Susanne Källgård, Erik Nygren, Jan Holmgren.
Construction and preclinical evaluation of mmCT, a novel mutant cholera toxin adjuvant that can be efficiently produced in genetically manipulated Vibrio cholerae
Vaccine 2016 34, 2121–2128, https://doi.org/10.1016/j.vaccine.2016.03.002

III. Manuela Terrinoni, Jan Holmgren, Michael Lebens and Maximilian Larena
Requirement for cyclic AMP/protein kinase A-dependent canonical NFκB signaling in the adjuvant action of Cholera Toxin and its non-toxic derivative mmCT
Frontiers in Immunology 2019 10:269.
doi: 10.3389/fimmu.2019.00269

IV. Manuela Terrinoni, Jan Holmgren, Michael Lebens and Maximilian Larena
Proteomic analysis of cholera toxin adjuvant-stimulated human monocytes identifies Thrombospondin-1 and Integrin-β1 as strongly upregulated molecules involved in adjuvant activity
Scientific Report 2019 9:2812
https://doi.org/10.1038/s41598-019-38726-

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ABSTRACT

Although most infections begin at a mucosal surface and may be prevented by effective vaccine stimulation of the local mucosal immune system, there are so far only a few mucosal vaccines available for human use. This thesis spans several areas that are important for future development of mucosal vaccines.

Future vaccine development will depend in part on the efficient production of recombinant antigens produced in bacterial expression systems. To avoid current problems with the use of antibiotics to maintain expression plasmids, an *E. coli* strain capable of producing recombinant proteins using vectors maintained without the need antibiotic was generated. The method is based on deletion of the essential *lgt* gene encoding a (pro)lipoprotein glyceryl transferase and complementing it with an expression vector carrying the non-homologous *lgt* gene from *V. cholerae*. A similar *V. cholerae lgt*-deleted strain was also constructed using the *E. coli lgt* gene for complementation. The strains had similar growth and production characteristics as their wild-type counterparts but maintained their expression plasmids without the need for antibiotics. The system was used to express two recombinant vaccine proteins, cholera toxin B subunit and a fusion protein for vaccination against atherosclerosis.

In the development of mucosal vaccines, it is often important to enhance immune responses using adjuvants, since most mucosally administered antigens are poorly immunogenic. Cholera toxin (CT) is the most powerful mucosal adjuvant known but is too toxic for human use. A mutated CT derivative (mmCT) was constructed and expressed in an engineered strain of *V. cholerae*. mmCT induced 1000 times less cAMP than native CT in a mouse thymocyte toxicity assay, was non-toxic in an infant mouse model and yet retained similar adjuvant properties as native CT. We suggest that mmCT is a promising candidate for use in future mucosal vaccines.

The mode of adjuvant action of mmCT and native CT was investigated using human and mouse antigen-presenting cells, which are primary target cells for adjuvants. Both molecules were found to activate cyclic AMP/protein kinase A-dependent canonical NF-κB signaling associated with inflammasome activation. The activation of these pathways was found to induce expression of two immunomodulatory proteins, THSB1 and ITGB1, as well as increased expression and activation of IL-1β, a cytokine which has been shown to play an important role for the adjuvant action of CT and mmCT.

Keywords: vaccine development, plasmid maintenance, Gram-negative bacteria, essential genes, complementation, Cholera Toxin, adjuvanticity, NF-κB, mmCT

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