Lipooligosaccharide and Cytolethal distending toxin of *Haemophilus ducreyi* and antibody responses

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Medicinaregatan 3, Göteborg.

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av

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Avhandlingen baseras på följande delarbeten:

I. **Xu T, Lundqvist A, Ahmed HJ, Ericsson K, Yang Y, Lagergård T**
Interactions of *Haemophilus ducreyi* and purified cytolethal distending toxin with human monocyte-derived dendritic cells, macrophages and $CD4^+$ T cells
Microbes and Infection 6 (2004) 1171-1181

II. **Lundqvist A, Kubler-Kielb J, Teneberg S, Ahlman K, Lagergård T**
Immunogenic and adjuvant properties of *Haemophilus ducreyi* lipooligosaccharides
Microbes and Infection 11 (2009) 352-360

III. **Lundqvist A , Fernandez-Rodrigez J K, Ahlman K and Lagergård T**
Haemophilus ducreyi cytolethal distending toxin and antibody responses; induction of specific antibodies in the genital tract
Submitted manuscript

GÖTEBORGS UNIVERSITET

LIPOOLIGOSACCHARIDE AND CYTOLETHAL DISTENDING TOXIN OF *HAEMOPHILUS DUCREYI* AND ANTIBODY RESPONSES

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ABSTRACT

The Gram-negative bacterium *Haemophilus ducreyi* causes chancroid, a sexually transmitted infection characterized by persistent ulcers on genitals. The disease is prevalent in developing countries and facilitates transmission and acquisition of HIV.

This thesis focuses on two bacterial virulence factors, lipooligosaccharide (HdLOS) and cytolethal distending toxin (HdCDT). The carbohydrate part of HdLOS is short and sialylated. HdCDT, an AB_2 toxin composed of three protein subunits, induces DNA double strand breaks, cause cell cycle arrest and death of target cells. Protective immunity against *H. ducreyi* is not well understood.

The general aim was to investigate the role of HdLOS and HdCDT in antibody responses, specifically to: 1) evaluate the function and viability of human monocyte-derived dendritic cells (DC), macrophages (MQ) and $CD4^+$ T-cells after interaction with *H. ducreyi* bacteria, HdLOS and HdCDT, *in vitro*; 2) define immunogenic and adjuvant properties of HdLOS; 3) evaluate the impact of HdCDT on the serum antibody responses and 4) define a procedure for the generation of high antibody levels to HdCDT in the genital tract, using a mouse model.

Bacteria and HdLOS stimulated an inflammatory cytokine response in the DCs and MQs, and activated cells induced $CD4^+$ T-cells to proliferate and secrete INF-γ. HdCDT caused apoptosis of DCs, inhibited the secretion of cytokines and intoxication resulted in failure of $CD4^+$ T-cells activation, *in vitro*.

Purified HdLOS is an immunogenic T-cell independent antigen. The majority of HdLOS antibodies was specific for the inner core and did not neutralize endotoxin activity. HdLOS possessed adjuvant properties and significantly increased the antibody response to proteins tested.

Active HdCDT is weakly immunogenic and induced low levels of specific antibodies. HdCDT did not down-regulate the antibody response to *H. ducreyi* antigens, despite the toxic activity on mouse immune cells, *in vitro*.

High levels of specific HdCDT antibodies in serum and genital tissue, including neutralizing antibodies, were induced by parenteral immunization of mice with formaldehyde detoxified HdCDT alone or in combination with aluminum salts or lipid A based adjuvants. The HdCDT toxoid can be evaluated as useful component of a vaccine against *H. ducreyi* and other CDT producing bacteria.

Keywords: *Haemophilus ducreyi*, lipooligosaccharide, cytolethal distending toxin
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