The role of IL-17A and IFNγ in vaccine-induced protection against *Helicobacter pylori*

Akademisk avhandling

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Avhandlingen baseras på följande arbeten:

I. **A double mutant heat-labile toxin from *Escherichia coli* LT(R192G/L211A), is an effective mucosal adjuvant for vaccination against *Helicobacter pylori* infection**
Sjökvist Ottsjö L, Flach C-F, Clements J, Holmgren J, Raghavan S
Infect Immun, 2013. ***81****(5): p. 1532-40*

II. **Defining the roles of IFNγ and IL-17A in inflammation and protection against *Helicobacter pylori* infection**
Sjökvist Ottsjö L, Flach C-F, Nilsson S, de Waal Malefyt R, Walduck A.K, Raghavan S
Submitted

III. **The role of IL-1 and IL-23 in inducing mucosal IL-17A responses against *Helicobacter pylori* infection in sublingually immunized mice**
Sjökvist Ottsjö L, de Waal Malefyt R, Raghavan S
In manuscript

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The role of IL-17A and IFNγ in vaccine-induced protection against *Helicobacter pylori*

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It is estimated that half the world's population is infected with *Helicobacter pylori* in the stomach. Chronic *H. pylori* infection can lead to peptic ulcer disease or gastric cancer, but only in a sub-population of infected individuals. Eradication of the bacteria with antibiotic treatment can be successful, but the emergence of antibiotic resistant strains of *H. pylori* is a problem in areas endemic with *H. pylori* infection. A mucosal vaccine would have the potential for boosting the immune response to *H. pylori*, preventing and thus reducing the prevalence of the infection. In spite of decades of intense research, no vaccine has yet been found to be effective against *H. pylori* infection in humans. The work in this thesis aimed to evaluate the impact of varying the adjuvant and route of mucosal vaccinations on the gastric immune response and protection against *H. pylori* infection in a mouse model. In particular, the role of cytokines induced by *H. pylori* infection was evaluated, with an overriding objective to separate the protective and pathogenic immune response in the stomach. In the first part of the thesis, the adjuvant effect of a detoxified mucosal adjuvant based on the *E. coli* heat labile toxin LT, double mutant heat-labile toxin R192G/L211A (dmLT) was evaluated. Furthermore, the thesis addressed the differences if any, in immune responses and protection against *H. pylori* infection after sublingual (SL; under the tongue) and intragastric (IG) route of vaccination with *H. pylori* antigens and the prototype mucosal adjuvant cholera toxin (CT). And finally, using gene knockout mice and neutralizing antibodies, the impact of cytokines IFNγ and IL-17A on bacterial load and immune responses was addressed.

Sublingual vaccination with *H. pylori* antigens and dmLT as an adjuvant was efficient in reducing the bacterial load in the stomach of mice, similar to when using the potent adjuvant CT, which is highly toxic in humans. Compared to infected unvaccinated mice, sublingual vaccination with dmLT enhanced stomach IL-17A and IFNγ secretion and proliferative responses to *H. pylori* antigens in mesenteric lymph nodes and spleen. Furthermore, we could show that there was a tendency for SL route to be more efficient than the IG route of vaccination in reducing the bacterial load in the stomach. And that the sublingual route of vaccination enhanced both IFNγ and IL-17A responses in the draining lymph nodes compared to unvaccinated mice. Studies on the role of individual cytokines in vaccine-induced responses revealed that after sublingual vaccination, IFNγ knockout ($IFN\gamma^{-/-}$) mice were protected against *H. pylori* infection and had elevated IL-17A production and lower inflammation scores in the stomach compared to vaccinated wild-type mice. Furthermore, neutralization of IL-17A in sublingually vaccinated $IFN\gamma^{-/-}$ mice abrogated protection against *H. pylori* infection. As IL-17A was found to be important for vaccine-induced protection, we next examined the mechanisms for induction and maintenance of IL-17A after sublingual vaccination by studying the role of cytokines IL-1β and IL-23. Our results show that after sublingual vaccination, IL-23, but not IL-1β, deficient mice were protected against *H. pylori* infection. Gastric IL-17A responses could not be induced after challenge in the absence of IL-1β, but could be maintained in the absence of IL-23.

In summary, we report that dmLT can be considered as a strong candidate mucosal adjuvant for use in a *H. pylori* vaccine in humans particularly when administered via the sublingual route. Furthermore, we show that IL-17A might contribute to protective immune responses, while IFNγ may promote inflammation. The results presented in this thesis will facilitate the design and administration of a vaccine against *H. pylori* infection in humans.

Keywords: *Helicobacter pylori*, vaccination, CT, dmLT, Sublingual, IFNγ, IL-17A, IL-1β and IL-23.

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