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Original article

Mucosal immune responses are related to reduction of bacterial colonization in the stomach after therapeutic *Helicobacter pylori* immunization in mice

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Abstract

The aim of this study was to investigate the capacity of oral and parenteral therapeutic immunization to reduce the bacterial colonization in the stomach after experimental *Helicobacter pylori* infection, and to evaluate whether any specific immune responses are related to such reduction. C57BL/6 mice were infected with *H. pylori* and thereafter immunized with *H. pylori* lysate either orally together with cholera toxin or intraperitoneally (ip) together with alum using immunization protocols that previously have provided prophylactic protection. The effect of the immunizations on *H. pylori* infection was determined by quantitative culture of *H. pylori* from the mouse stomach. Mucosal and systemic antibody responses were analyzed by ELISA in saponin extracted gastric tissue and serum, respectively, and mucosal $CD4^+$ T cell responses by an antigen specific proliferation assay. Supernatants from the proliferating $CD4^+$ T cells were analyzed for Th1 and Th2 cytokines. The oral, but not the parenteral therapeutic immunization induced significant decrease in *H. pylori* colonization compared to control infected mice. The oral immunization resulted in markedly elevated levels of serum IgG+M as well as gastric IgA antibodies against *H. pylori* antigen and also increased *H. pylori* specific mucosal $CD4^+$ T cell proliferation with a Th1 cytokine profile. Although the parenteral immunization induced dramatic increases in *H. pylori* specific serum antibody titers, no increases in mucosal antibody or cellular immune responses were observed after the ip immunization compared to control infected mice. These findings suggest that *H. pylori* specific mucosal immune responses with a Th1 profile may provide therapeutic protection against *H. pylori*.

Keywords: T cell response; *Helicobacter pylori*; Mouse model; Therapeutic immunization; Parenteral/oral immunization; Cholera toxin; Mucosal immunity; Alum

1. Introduction

Helicobacter pylori colonizes the human gastric and duodenal mucosa, where it can give rise to chronic gastritis, peptic ulcer and gastric cancer in a subpopulation of individuals [1]. The current treatment against *H. pylori* infection consists of a combination therapy with two different antibiotics together with a proton-pump inhibitor, which is in most cases successful in eradicating the bacteria and healing ulcers [2]. However, there are some major drawbacks with such therapy, including high cost, poor patient compliance and increased risk of developing antibiotic resistance, making it unsuitable for use in, e.g. the developing world [3]. Furthermore, such treatment does not protect against reinfections, which often occur in developing countries. Vaccination would therefore be a suitable alternative or complement to antibiotic treatment to eradicate the bacteria and also to prevent reinfection. A vaccine can be administered either prophylactically or therapeutically. A prophylactic vaccine should be given to children before they become infected, since most infections

Abbreviations: APC, antigen presenting cells; CFU, colony forming units; CT, cholera toxin; DC, dendritic cells; ELISA, enzyme linked immunosorbent assay; *H. pylori*, *Helicobacter pylori*; IFN-γ, interferon-gamma; Ig, immunoglobulin; IL-, interleukin; ip, intraperitoneal; MHC, major histocompatibility complex; MLN, mesenteric lymph nodes; MP, membrane protein preparation; SS1, Sydney strain 1; Th, T-helper; TNF-α, tumor necrosis factor-alpha.

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occur during early childhood [4]. However, previous studies from our laboratory have shown that immune responses to a model vaccine may be readily induced in the stomach of *H. pylori* infected but not uninfected individuals [5] suggesting an advantage of therapeutic vaccination over prophylactic immunization. Furthermore, in experimental animals, therapeutic oral immunization has been shown to protect not only against the primary infection but also against reinfection [6,7]. Nevertheless, most immunization studies against *H. pylori* infection in mice have used a prophylactic immunization regimen. In addition, it has been shown that both the oral and the parenteral route can induce significant decrease in bacterial load in the stomach compared to control infected mice after prophylactic immunization against *H. pylori* in mice [8,9].

Several studies have tried to elucidate the mechanisms leading to reduction in bacterial load after primarily prophylactic and to a lesser extent after therapeutic immunization. Systemic antibodies, such as serum IgG, do not seem to be associated with decreased bacterial density or eradication of *H. pylori* whereas the role of mucosal antibodies is less clear. The importance of T cell responses in prophylactic protection against *H. pylori* infection has been evaluated using gene knockout mice, showing that $CD4^+$, but not $CD8^+$ T cells are required for vaccine induced protection [10]. Furthermore, the importance of $CD4^+$ T cells, particularly of the Th1 type, for the reduction of *H. pylori* colonization in the stomach after oral prophylactic immunization has been emphasized [11].

For the design of an effective candidate vaccine against *H. pylori* infection, immune markers predicting the outcome of an immunization regimen (sterilizing protection or reduction in bacterial load in the stomach) need to be defined in a systematic manner, particularly after therapeutic immunization. In this study we have compared the bacterial colonization in the stomach of *H. pylori* infected mice after oral and systemic therapeutic immunization, respectively, using immunization strategies that have previously been shown to be effective for prophylactic immunization. We have also addressed the question whether systemic and/or mucosal immune responses can be used as markers to predict protection against an ongoing *H. pylori* infection in mice.

2. Material and methods

2.1. Animals

Six to eight week old C57BL/6 female mice were purchased from B&K Universal (Sollentuna, Stockholm, Sweden) and housed in microisolators at the Laboratory for Experimental Biomedicine, Göteborg University, Sweden, during the study. All experiments were approved by the Ethical Committee for Laboratory Animals in Göteborg.

2.2. Bacteria and culture conditions for infection

Mouse-adapted *H. pylori* Sydney strain 1 (SS1) ($CagA^+$, $VacA^+$, Le^y) [12], was prepared for infection as previously described [7].

2.3. H. pylori antigen preparations

Lysate of *H. pylori* bacteria, strain Hel 305 ($CagA^+$, $VacA^+$, Le^x), a clinical isolate from our strain collection, was prepared as described [6]; the protein content was determined by measuring the absorbance. The antigen preparation was stored in aliquots at –70 °C until further use. Whole membrane protein (MP) was prepared from *H. pylori* strain SS1 and 305 as previously described in detail [13].

2.4. Infection and immunization

Female C57BL/6 mice were orally infected with approximately 3×10^8 colony forming units (CFUs) of *H. pylori* SS1 bacteria in Brucella broth under anesthesia (Isoflurane; Abbott Scandinavia Ab, Solna, Sweden) as previously described [14]. Two weeks after infection the mice were immunized intragastrically or intraperitoneally (ip). The intragastric immunizations (which will be referred to as oral immunization throughout the paper) were either given twice with a 2 week interval or given as 4 weekly doses of 400 μg of lysate in 300 μl 3% sodium hydrogen carbonate buffer together with 10 μg cholera toxin (CT; List Biological Laboratories Inc, Madison, NJ, USA). The ip immunizations were given twice with a 2 weeks interval with 100 μg lysate in 100 μl of 1:1 solution of PBS and Alum (Imject Alum, Pierce, Rockford, IL, USA) as adjuvants as previously described [15]. Some mice were left untreated as infection controls. Also, some mice were immunized ip prophylactically with alum and lysate, as previously described [15].

2.5. Quantitative culture of H. pylori SS1 from the stomachs of mice

Infected and immunized mice were killed 2 weeks after the last antigen administration together with control infected mice. One half of the stomach was homogenized in 2 ml Brucella broth with a tissue homogenizer (Ultra Turrax; IKA Laboratory Technologies, Staufen, Germany). Serial dilutions of the homogenate were thereafter plated on Skirrow blood agar plates as previously described [7].

2.6. Saponin extraction

Immunoglobulin (Ig)A was extracted from the stomach and small intestine using saponin extraction of the tissues. Briefly, the organs were excised and weighed before storage at –70 °C in a PBS solution containing 2 mM phenylmethylsulfonyl fluoride, 0.1 mg/ml of trypsin inhibitor from soybean (Sigma), and 0.05 mM EDTA. The tissue samples were thawed and then permeabilized with saponin (Sigma) at a final concentration of 2% (w/v) in PBS at 4 °C over night. The tissue samples were then centrifuged at 13,000 × *g* for 10 min and the supernatants were analyzed for total and specific IgA antibodies by Enzyme linked immunosorbent assay (ELISA).