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Expression of Urease B from Iranian Helicobacter pylori in Apoplast of Tobacco

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Abstract

Helicobacter pylori (H. pylori) are established as the etiologic agent of chronic active gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue lymphoma. The development of a vaccine against H. pylori has become a priority to prevent and cure H. pylori infection in scientific societies. The UreB (urease B) subunit is the most effective and common immunogen of all strains of H. pylori and can stimulate the immune response protecting the animal and human body against the challenge of H. pylori. In this study this antigen produced with apoplastic signal peptide from extensin protein of carrot in tobacco. Expression assessment in transcriptome levels revealed significant difference among the transgenic lines well and also protein assay through indirect ELISA With human polyclonal antiserum demonstrated that the average of expressed UreB protein accounted for 3/2mg/kg that was 0/1% from total proteins that exist in apoplastic space. These results showed that utilization of carrot extension signal peptide can be an efficient method for increasing of percentage of recombinant protein among total soluble protein and because of lower protein contamination can decrease downstream expense of protein purification.

Helicobacter pylori infection is a major risk factor for chronic gastritis, digestive ulcers, gastric adenocarcinoma and lymphoma. Due to the decreasing efficacy of anti- H. Pylori antibiotic therapy in clinical practice, there is renewed interest in the development of anti-Pylori vaccines. Bacillus subtilis is non-pathogenic and can produce endospores, which can survive under extreme conditions. These features make the B. subtilis spore an ideal vehicle for delivery of heterologous antigens to extreme environments such as the gastrointestinal tract. In this study, we displayed H. pylori urease B protein on the B. subtilis spore coat using the Spore coat protein CotC as a fusion partner. Western blot analyses were used to verify urease B surface expression on spores. Recombinant spores displaying the urease B antigen were used for oral immunization and were shown to generate humoral response in mice. Urease B-specific secretory IgA in faeces and IgG in serum reached significant levels 2 weeks after oral dosing. In addition, oral immunization of recombinant urease B spores induced a significant reduction (84 %) in the stomach bacterial load (0.25±0.13×10(6) c.f.u.) compared to that in the non-recombinant spores treated group (1.56±0.3×10(6) c.f.u.; P<0.01). This report shows that urease B expressed on B. subtilis spores was immunogenic, and oral administration of urease B spores can provide protection against H. pylori infection.

The use of Lactococcus lactis as an antigen delivery vehicle for mucosal immunization has been proposed. To determine whether L. lactis could effectively deliver Helicobacter pylori antigens to the immune system, a recombinant L. lactis expressing H. pylori urease subunit B (UreB) was constructed. Constitutive expression of UreB by a pTREX1 vector resulted in the intracellular accumulation of UreB to approximately 6.25% of soluble cellular protein. Five different oral regimens were used to vaccinate C57BL/6 mice and the immune response measured. One regimen, which consisted of four weekly doses of 10(10) bacteria, followed after an interval of approximately 4 weeks by three successive daily doses, was able to elicit a systemic antibody response to UreB in the mice, although subsequently, a similar regimen produced a significant antibody response in only one out of six mice. The other three regimes, in which mice were vaccinated with two or three sets of three consecutive daily doses of recombinant bacteria over 30 days, failed to elicit significant anti-UreB serum antibody responses. In three regimens, the immunized mice were then challenged by H. pylori strain SS1 and no protective effect was observed. These findings suggest that any adjuvant effects of L. lactis are unlikely to be sufficient to produce an effective immune response and to protect against H. pylori challenge, when used to deliver a weak immunogen, such as UreB.

Helicobacter pylori are a pathogen involved in gastric diseases such as ulcers and carcinomas. H. pylori's urease is an important virulence factor produced in large amounts by this bacterium. In previous studies, we have shown that this protein is able to activate several cell types like neutrophils, monocytes, platelets, endothelial cells, and gastric epithelial cells. Angiogenesis is a physiological process implicated in growth, invasion and metastization of tumors. Here, we have analyzed the angiogenic potential of H. pylori urease (HPU) in gastric epithelial cells. No cytotoxicity was observed in AGS, Kato-III, and MKN28 gastric cell lines treated with 300 nM HPU, as evaluated by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As we previously reported in neutrophils, treatment with 300 nM HPU also had an anti-apoptotic effect in gastric epithelial cells leading to a 2.2-fold increase in the levels of Bcl-XL after 6 h, and a decrease of 80% in the content of BAD, after 48 h, two mitochondrial proteins involved in regulation of apoptosis. Within 10 min of exposure, HPU is rapidly internalized by gastric epithelial cells. Treatment of the gastric cells with methyl-β-cyclodextrin abolished HPU internalization

suggesting a cholesterol-dependent process. HPU induces the expression of pro-angiogenic factors and the decrease of expression of anti-angiogenic factors by AGS cells. The angiogenic activity of HPU was analyzed using in vitro and in vivo models. HPU induced formation of tube-like structures by human umbilical vascular endothelial cells in a 9 h experiment. In the chicken embryo chorioallantoic membrane model, HPU induced intense neo-vascularization after 3 days. In conclusion, our results indicate that besides allowing bacterial colonization of the gastric mucosa, H. pylori's urease trigger processes that initiate pro-angiogenic responses in different cellular models. Thus, this bacterial urease, a major virulence factor, may also play a role in gastric carcinoma development.