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Spore-based vaccine against *Helicobacter pylori* infection

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Abstract

Helicobacter pylori infection is considered as the most prevalent bacterial infection worldwide. Although in most cases it doesn't cause any symptoms, in about 10% of infected people it leads to the development of gastric or duodenal ulcers and in 1-2% it can cause a gastric cancer. The treatment, which consists of combination of two antibiotics and a proton pump inhibitor, usually is effective but relatively expensive, long and causing a lot of side effects. Moreover, the number of antibiotic resistant strains is still increasing. However, the most significant drawback of this therapy is its failure to prevent reinfection. That's why it is necessary to look for new forms of therapy, especially those that can provide a permanent immunity. A major problem regarding the studies on the vaccine is little knowledge about the immune response to the infection and about the mechanism underlying the protective response.

Novel subunit vaccines use purified recombinant antigens instead of whole, inactivated or attenuated, bacterial cells. Thanks to this they can be used without any side effects. However purified proteins are not stable when stored and administered to the patient. They are also not effective enough in eliciting a strong immune response. Hence there is a need to use a proper antigen carrier and a proper adjuvant to enhance the immune response.

Development of methods for oral immunization is an interesting alternative to current vaccines, which require subcutaneous administration. The new route of administration of the vaccine may not only facilitate its use in the developing countries or limit the psychological barrier (a fear of needles) but also provides an opportunity to stimulate the local immune response at the site of administration (digestive system), in cases when the aim is to protect against pathogens invading trough the gastrointestinal tracts.

The spores of non-pathogenic, probiotic bacterium *Bacillus subtilis* can be used as a durable and convenient to use carrier for heterologous proteins such as enzymes used in the industry, therapeutic proteins or antigens able to induce a specific immune response. In this work I presented the construction of four vectors which allow to display heterologous protein on the spore surface in the form of fusions with coat protein. Thanks to them it is possible to create an N- or C-terminal fusion, as well as to separate the fusion partners with a short peptide linker.

In the next part of this study I examined an immune response of mice to the recombinant spores presenting two model antigens of *H. pylori*: UreA and UreB. Spores were administered orally or intranasally. In addition, I studied the effect of adjuvants, such as chitosan, saponins, aluminum hydroxide and interleukin 2 presented on the spore surface, on the modulation of the immune response. IgG levels were determined in the serum of immunized mice, as well as the presence of sIgA

antibodies in the digestive system and the feces. The profile of cytokines secreted by antigen-stimulated splenocytes obtained from immunized mice were also determined.

The results showed that the recombinant spores presenting the *H. pylori* antigens administered orally with adjuvant (aluminum hydroxide and spores presenting IL-2) induce Th1/Th17-polarized immune response. Spores given intranasally induce a balanced Th1/Th2/Th17 immune response and lead to an increase in the level of antigen-specific IgG antibodies in the serum. Furthermore, *in vitro* studies using cell lines have shown that cytokines secreted by the splenocytes obtained from the immunized mice strongly activate murine macrophages.

The results presented in this work suggest that *B. subtilis* spores are a promising antigen carrier for mucosal vaccines against *H. pylori* infection. However, it is necessary to conduct further research in order to select the appropriate antigens and adjuvants, as well as to assess the effectiveness of potential vaccines in challange experiments.