A recombinant *Bacillus subtilis* vaccine to induce CD8$^+$ T cell response

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Background and objective

Cytotoxic CD8+ T cells (CTLs) play an important role in eradication of many intracellular pathogens, as well as in destruction of cancer cells. Interaction of the T cell receptor of the lymphocytes (TCR) with major histocompatibility complex class I molecules (MHC class I) located at the surface of the antigen presenting cells (APCs), such as dendritic cells (DCs) or macrophages, provide specificity of the interaction between those two types of cells. After recognizing the MHC I – peptide complexes of infected cells and receiving costimulation from the CD4+ T lymphocytes, CTLs become activated, what leads to killing of the target cells by apoptosis and release of membrane perforating granzymes and perforins. More importantly, a population of memory T cells is generated, thereby leading to resistance to a pathogen, antigen of which was presented. Different strategies can be used to deliver antigens to the cytosol of APCs, among which use of the bacterial carriers constitutes probably the most studied and the most promising strategy. Both attenuated and commensal microorganisms are used in the next generation vaccine design. However, due to the potential risk of conversion to a virulent strain and causing infection, pathogenic vectors are less likely to use. A major drawback of the systems based on non-pathogenic bacteria is the lack of invasiveness, so the antigen delivery could be less effective than in case of pathogenic bacteria. To overcome this problem, virulence determinants of intracellular pathogens could be expressed in non-virulent species. Among proteins, which activity leads to bacterial invasion and escape from the vacuole to the cytoplasm of the host cell, there are virulence determinants such as listeriolysin O (LLO) of an intracellular pathogen, *Listeria monocytogenes*. Interestingly, LLO is simultaneously a major virulence factor and a major immunogen, what makes it an ideal adjuvant candidate [1].

The aim of this project was construction of a series of bacterial delivery vectors based on the non-pathogenic, model Gram-positive microorganism *Bacillus subtilis*, as well as evaluation of their potency to deliver different variants of the model antigen, chicken egg ovalbumin (OVA), to the cytosol of dendritic cells of JAWS II line. Along with examining the presentation of the OVA antigens in the MHC I complexes, ability to induce the response of cytotoxic T cell lymphocytes was evaluated.

Materials and methods

B. subtilis strains producing recombinant fusion antigens were obtained by standard methods of molecular cloning. All constructed delivery vectors expressed listerial LLO toxin enabling escape from eukaryotic vacuole and consequently facilitating heterologous production of immunogenic proteins in the cytosol of APCs [2]. Ability to invade eukaryotic cells, as well as cytotoxicity and haemolytic activity were examined. Presentation of the OVA epitope in the context of the MHC I molecules was evaluated by flow cytometry after staining the *Bacillus*-infected DCs with 25-D1.16 antibody recognizing H-2K^b-OVA$_{257\text{-}264}$ complexes. For evaluation of *B. subtilis* ability to elicit T cell activation, proliferative response of CFSE-labelled cytotoxic CD8+ T cells OT-I isolated from transgenic murine lymph nodes was determined by flow cytometry [3].

Results and conclusions

Tests conducted during the described project revealed that *B. subtilis* strain 1009_1 producing whole-length OVA antigen fused to N-terminal sequence of LLO is most capable to induce the presentation of the OVA antigen on the surface of APCs and to activate response of cytotoxic T cells. The comparison to strains producing different variants of LLO-OVA antigens indicated that 1009_1 strain is the most promising candidate for composition of the universal antigen-delivery vector.

Keywords: antigen delivery; *Bacillus subtilis*; CTL; MHC class I

References

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