Abstract

C. difficile is a Gram-positive bacterium, which infections are of increasing concern, affecting global health and economy. Thanks to its ability to sporulate, significant part of the population is contaminated with spores of this bacterium. However, in normal conditions, the infectious process does not occur. Infection caused by *C. difficile* (CDI) is mostly connected with prolonged antibiotic treatment, which disrupts indigenous gut microbiota, leaving the intestine susceptible to colonization by outgrown vegetative cells of *C. difficile*. Following colonization of host gastrointestinal tract (GIT), the process of toxin production begins. Clostridial toxins A and B are the factors responsible for the appearance of disease's symptoms, which range from self-limited diarrhoea to more severe pseudomembranous colitis (PMC). Complications of PMC comprise colon perforation and toxic megacolon, which may pose a life-threat. Nowadays, the leading therapy for the treatment of this infection consists of discontinuing the ongoing antibiotic therapy and its possible replacement with more specific antibiotics, such as metronidazole and vancomycin. However, a larger amount of cases connected with decreased sensitivity of *C. difficile* to respond to traditional therapy is being reported. In this scenario, the absence of an efficient alternative therapy requires the development of new strategies to fight these infections. Mucosal vaccination has been reported to be an efficient strategy to induce active immunization against pathogens that begin the infectious process primarily at the mucosal surface. However, only few studies succeeded in obtaining a functional mucosal vaccine, due to difficulties encountered in antigen delivery to the mucosal immunization sites. Indeed, purified antigens do not survive the extreme conditions found in the stomach, resulting in poor and therefore inefficient induction of immune response.

In the last 15 years, *B. subtilis* endospores have been described as an ideal tool for antigen surface display. Thanks to the resistance properties of spores, displayed antigen is protected from the harsh gastrointestinal environment. In line with this idea, the present work aimed to construct an edible vaccine against PMC based on recombinant strains of *B. subtilis* presenting a *C. difficile* antigen on spore surface. In order to reach this goal, FliD protein of *C. difficile* was chosen for being exposed on spore surface as a fusion protein with proteins of the coat layer. The choice of FliD was made based on previous reports, which assessed its immunogenicity and its involvement in the colonization process. For this reason, the induction of efficient immune response toward FliD is believed to result in prevention of pathogen's colonization. Preventing the colonization would correlate with reduction of carriage of the pathogen, resulting in diminished individual-to-individual cross-contamination and recurrence of CDI. In the first part of the project, several *B. subtilis* recombinant strains were created, presenting the fusion of FliD with various coat proteins. In addition, strains carrying the double fusion consisting of coat protein, FliD and a fragment of IL-1β were created. Furthermore, as demonstrated that *B. subtilis* spores are able to germinate in the gastrointestinal environment, recombinant strains of *B. subtilis* able to produce FliD protein during the vegetative growth were prepared. The second part of the project consisted in assessment of immune response in laboratory animals induced by administration of prepared FliD presenting spores through the mucosal route. Five immunization experiments on BALB/c mice were conducted to evaluate the induction of the immune response deriving from immunization with FliD presenting spores. Additionally, the immunization experiments were designed in order to evaluate the possible enhancement of immune response elicited by production of FliD during vegetative growth or by addition of immunomodulators, such as IL-1β and IL-2, presented on spore surface as a heterologous component of the spore coat. The last immunization experiment was conducted with the aim to evaluate the differences between intragastric and intranasal route of administration. Simultaneously, an investigation aiming to evaluate the differences in inducing the immune response between the recombinant and non-recombinant approach to display

FliD on spore surface was conducted. Samples taken from sacrificed mice were analysed for the profiling of the immune response elicited by FliD presenting spores.

Intragastric administration of FliD presenting spores failed to induce humoral specific response against FliD. However, an induction of Th1-mediated immune response in case of administration with BAN05 (CotB-linker-FliD) could be observed, while BAN03 (CotG-FliD) succeeded in inducing a pro-inflammatory Th17 immune response. Addition of immunomodulators or production of FliD during vegetative growth did not significantly increase the immunogenic potential of the formulation. On the other hand, intranasal immunization succeeded in inducing a strong humoral immune response. Such result was observed for the FliD presenting spores prepared with non-recombinant approach.

In conclusion, immunization with recombinant spores led to induction of cellular and inflammatory response, but not humoral. Intranasal administration was demonstrated to be superior to intragastric, especially for non-recombinant approach, which more likely may confer protection to CDI than recombinant one. However, in order to efficiently evaluate whether the profile of immune response observed is correlated to induction of protection against CDI, experiments of challenge with infectious bacteria should be conducted.

Results obtained confirmed that FliD presenting spores possess the potential for being used as vaccine against PMC, but there is a need to improve the immunogenic potential of such vaccine formulation.

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