Abstract

Influenza virus infections cause considerable morbidity and mortality in the world. Current immunization against influenza is provided using parenterally given influenza vaccines. These vaccines can induce good systemic immunity but they fail to induce a protective mucosal immunity. Because of persisting threat of new highly pathogenic influenza A subtypes development, a vaccination inducing intersubtypic cross-protection is desirable. The principal aims of the present study were: firstly, to explore the effect of *Bacillus firmus* (BF) and its delipidated form (DBF) as mucosal adjuvants for immunization via respiratory tract, secondly to test the potencial of BF and DBF to induce intrasubtypic and intersubtypic protection and finally to characterize the mechanism of adjuvant effect.
The adjuvant effect of BF and DBF was tested after intratracheal or intranasal immunization of mice with inactivated influenza virus type A or B. Both types of immunization stimulated both systemic and mucosal immunity. Inactivated influenza virus type B was less immunogenic in contrast to type A. Adjuvant immunization with mixture of virus (type A or B) + DBF increased both systemic and mucosal antibody response. The effect of BF and DBF on induction of heterosubtypic immunity was tested in *in vivo* protective experiments. After immunization, mice were infected with influenza A (A/PR/8/34) or B (B/Lee/40), both lethal for mice. Our experiments documented a pronounced protective effect of the adjuvant immunization against homologous virus and a conspicuous cross-protection (protection against H1N1 after immunization with H3N2 and protection against B/Lee after immunization with B/Yamanashi). The mechanism of adjuvant effect was tested in NALT after intranasal immunization of mice by inactivated influenza virus type A, adjuvant alone (DBF) and by mixture of virus+DBF. We tested the expression of selected genes for cytokines, toll-like receptors and other genes participating in immune response by qPCR. Intranasally given DBF and mainly mixture virus+DBF induced expression of cytokines characteristic for Th1 immune response (IFN-γ and IL-2) whereas expression of genes characteristic for Th2 was decreased (IL-4). Increased expressions of IL-6 and IL-10 are important for production of IgA. Differences in expression of TLR7, TLR9, CCR7 and type I IFN followed by PCA analysis indicate activation of plasmacytoid dendritic cells (pDC). DBF has been shown to be very efficient adjuvant for stimulation of both mucosal and systemic immune responses, in induction of heterosubtypic immunity against influenza virus and in stimulation of innate immunity.