**Abstract**

Influenza is a serious illness worldwide, causing high morbidity and mortality. 10-20% of world population fall ill with influenza each year and 250 000 - 500 000 people die annually. The most efficacious protection to date is vaccination. Current vaccines are efficient only one season because of fast mutation rate of influenza virus. The effort to create an effective vaccine faces lack of potent adjuvant, which can adequately stimulate and modulate immune system to protect organism from virus infection. Moreover, today’s vaccines administered parenterally do not induce immune response on mucosal surfaces. *Bacillus firmus*, a Gram-positive non-pathogenic bacterium, has strong immuno-modulating properties and is able to induce cross-protection when administered with influenza virus antigens. Immunization with *Bacillus firmus* stimulates production of neutralizing antibodies, but other mechanisms of its action remain to be elucidated.

To better understand the mechanisms how is antiviral immunity enhanced by *Bacillus firmus* (delipidated fraction, DBF), the effect of immunization with DBF only was studied on mouse model. In last decade it has become obvious that intranasal immunization can induce both systemic and mucosal immune response and in case of influenza it can induce cross-protection. Therefore we used intranasal immunization in our study and monitored immune response in nasal-associated lymphoid tissue (NALT).

Production of IFN-γ in NALT and spleen was measured by flow cytometry. IFN-γ is considered to be main regulator of immune response during immunization or infection of airways with virus. However, one dose of DBF had no effect on production of IFN-γ in NALT and spleen. Repeated administration of DBF induced secretion of IFN-γ 7 days after second administration in both NALT and spleen. Effect of immunization with DBF on population of T regulatory lymphocytes (Treg) and production of IL-10 in NALT and spleen was also studied. Immunization with DBF had no effect on overall population of Treg and also on proportion of thymic-derived Treg and peripherally-induced Treg. Production of IL-10 by T lymphocytes was low, in NALT it was not even detectable.

Intranasal immunization can influence even distant mucosal sites, such as small intestine. Therefore we studied gene expression in small intestine after intranasal immunization with DBF using PCR method. We analyzed gene expression for markers of Th1, Th2, Th17 and Treg polarization of immune response. Nevertheless, even repeated administration of DBF did not induce significant changes in gene expression of cytokines and other markers in intestine cells.

Administration of one dose of DBF had no effect on secretion of IFN-γ by T lymphocytes, proportion of Treg or gene expression in small intestine. Repeated immunization induced production of IFN-γ both in NALT and spleen. In conclusion, repeated intranasal immunization
with DBF had immunomodulating effect on both mucosal and systemic immunity in case of Th1 polarization, which is important for immune response against influenza and other viral or bacterial infections.