Immune response of mice after mucosal immunization by influenza virus type A with bacterial adjuvant *Bacillus firmus*

Immunization of mice by inactivated influenza virus via respiratory tract induces a good mucosal and systemic immune response if bacterial adjuvant - delipidated G^+ non-pathogenic bacterium *Baccillus firmus* (DBF) - is used. BALB/c mice were immunized intratracheally (IT) or intranasally (IN) with inactivated influenza A/PR/8/34 virus in combination with adjuvant DBF (50, 100, 200 or 500μg per immunization dose). We tested the production of antibodies against homologous virus and cross-reacting antibodies against subtype H3N2, H6N2 and H9N2 viruses in serum and mucosal secretions of nose, lungs and intestine by the ELISA method. Immunization of mice with virus itself induces the production of antibodies against homologous virus and lower production of cross-reacting antibodies against heterologous subtypes. Immunostimulatory adjuvant activity (optimal 100μg per immunization dose) enhances systemic and mucosal antibody production against homologous virus (H1N1) and markedly against heterologous subtypes (H3N2, H6N2, H9N2), especially after IT immunization of mice.

For evaluation of cellular immunity, we tested spleen cell proliferation of immunized mice by ^{3}H-thymidine incorporation and cytokine production in culture supernatants by the ELISA after the specific stimulation of splenocytes *in vitro*. We found out that adjuvant immunization reduces the immunosuppressive effect of the virus on cell proliferation in culture and affects the production of cytokines. Adjuvant immunization increased IFN-γ and IL-4 production. IFN-γ stimulation was more pronounced, which shows the Th1 supporting effect of the adjuvant.

Keywords: influenza A virus, intratracheal immunization, intranasal imunization, bacterial adjuvant, heterosubtypic immunity, mucosal immune response, systemic immune response, antibodies, cytokines, ELISA, blast transformation