WITH INFLUENZA VIRUS

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Introduction. Vaccination is a major tool to protect people from seasonal infections of different strains of influenza virus that presently infects millions of individuals worldwide. Virus genome is highly polymorphic, and universal vaccine that protects against permanently changing virus is still under development. Despite notable differences between humans and rodents in the disease course, immunobiology and clinical evaluations, murine infectious models remain one of the major tools to test approaches for influenza vaccine development.

Materials and methods. BALB/c mice were subcutaneously immunized with 8-22-mer peptides or alternatively with peptide conjugated to KLH in complete Freund's adjuvant (CFA), and then received second immunization in three weeks with peptides and/or peptide~KLH in incomplete Freund's adjuvant (collaboration with the Institute of Microbiology and Virology, Kiev, Ukraine). In two weeks, titers of peptide-specific antibodies were measured in mouse serum. Splenocytes were in vitro stimulated with peptides for two days, and production of IFN-gamma, TGF-beta, IL-2, and IL-4 was assayed by ELISA. To test protective effect of mouse vaccination, white mice received two immunizations with a cocktail of selected peptides (at the Research Institute for Biological Safety Problems, Zhambulskaya oblast, Gvardeisky, Kazakhstan). Then mice were infected intranasally with pandemic strain A/California/7/09 (H1N1) or with strain A/domestic goose/Pavlodar/1/05 (H5N1). Virus titers in lungs, body weight, mouse lethality, and histopathological evaluation for liver necrosis and lung infiltration were scored and analyzed.

Results and discussion. Previously, we selected 53 peptides of the virus proteome using bio-informatics approaches and filtered peptides for sequence conservancy and recognition by HLA of class I and II. After mice immunization with groups of pooled peptides, significant immune responses were detected for all measured cytokines and serum antibodies. Based upon immune response screening, we selected six peptide groups that altogether stimulated strong anti-peptide antibody IgGAM production, and high peptide-specific IL-2, IL-4, and IFN-gamma responses, while TGF-beta response was zeroed to prevent immunosuppression. These six peptides represent hemagglutinin (362-410 aa), M2 proton channel (31-54 aa) and neuraminidase (21-38 aa). At the next step, we immunized mice with a cocktail of six selected peptides in hope to protect mice against the acute infection with influenza. Based on body weight dynamics, mice perfectly tolerated two immunizations with selected peptides. In vitro experiments confirmed no toxicity of selected peptides even at a high concentration for mammalian cells. Peptide cocktail demonstrated a significant protective effect upon mouse survival during infection with H5N1 strain of virus ($p < 0.02$ Student's t-test). This protective effect correlated with significantly lower titers of the virus in mouse lungs when compared to mice immunized with PBS-adjuvant ($p < 0.019$).

Conclusions. We found combination of several conservative epitopes of viral proteome that ensure partial protection of rodents from otherwise killing concentration of the virus.

Acknowledgments. This study was supported by a grant from Ministry of Education and Science, Republic of Kazakhstan.