Abstract for general public

Despite massive vaccination programmes, Hepatitis B Virus (HBV) remains the major cause of liver diseases. Estimated 2 billion people worldwide suffer from hepatitis B, including over 250 million with chronic hepatitis B (CHB), and 600 thousand patients die annually from CHB complications, mostly in developing countries. Therefore, more effective but at the same time economically feasible and commonly available preventive vaccines and **CHB therapeutic vaccines, are particularly welcome**.

Commonly used, yeast-produced 2nd-generation preventive vaccines are based on the small surface antigen (S-HBsAg). More effective are the 3rd-generation vaccines containing also the medium and/or large surface antigens (M/L-HBsAg). Yet, they are produced in costly mammalian cells, thus their use is limited. The main component of tested CHB therapeutic vaccines is **the core antigen (HBcAg), inducing strong Th1 response - mostly cellular type, which can be supplemented with HBsAgs or their immunodominant domains - inducers of Th2 response - humoral type**. Virus-Like Particles (VLPs) VLPs based on HBcAg are well-characterized potential vaccine templates. Antigens or their fragments (epitopes) loaded on the surface of VLPs are therefore presented in a highly ordered structure, thus effectively for the immune system.

VLP-assembled plant-derived antigens are considered alternative vaccines, due to low cost, biosafety, bioactivity, and possibility of oral immunisation. Immunogenicity of plant-produced S-HBsAg or HBcAg has been already proved. Both antigens, when used in injection immunization or that comprising injection priming and oral boosting, induced significant response of proper polarization. **These results indicate that key epitopes of both antigens, combined in one type of chimeric VLPs, can induce the immune response of mixed Th1/Th2 polarization, required for CHB therapy**. However, both production and immunogenicity of plant-derived chimeric VLPs need to be studied. Although expression of HBcAg was efficient, assembly of HBcAg-based chimeric VLPs is still in preliminary phase.

The **main goal** of this project is **the development and the determination of immunogenicity of novel types of chimeric VLPs - formed by plant-produced HBcAg displaying key HBsAg epitopes**: aSHBs and ΔpreS1 as well as TransLocation Motif (TLM) – enhancing permeability and immunogenicity of VLPs. In details – study is aimed to explain mutual effects of HBcAg and HBsAg epitopes, together with impact of TLM and mosaic structure of VLPs on the efficacy and type of evoked immune response.

This project will be realized in **consecutive milestones**:

- Obtaining spectrum of VLPs of two types: 'monoantigenic'- assembled exclusively by one type of monomers, i.e. HBcAg with fused aSHBs or ΔpreS1 epitope, and 2) mosaic, assembled by unaltered HBcAg and HBcAg-epitope, both types including variants with TLM motif.
- VLPs expression in *Nicotiana benthamiana* plants by transient transformation. This expression system characterizes: 1) high levels of protein expression with possibility of heteromultimeric VLPs production, 2) scalability and low cost, 3) fast expression time, 4) biosafety
- Purification and quantification of VLPs expressed, followed by comprehensive structural analysis.
- Immunogenicity determination of chimeric VLPs in animal (C57BL/6 mice) trials. Complex characterization of humoral and cellular immune response including: assay of serum antibodies; circulating white blood cells; determination of subpopulations of splenocytes and cytokine production will enable evaluation of proportion of Th1 and Th2 polarizations.

Optimized production of chimeric VLPs together with determination of the immune response type will bring new data on the interaction effect of different types of epitopes, as well as have potential implications for **immunology, prophylaxis, therapy**, other **medical applications** and plant biotechnology. The results of the project will constitute indispensable basis before studies on further development of vaccination and studies on therapeutic effects of developed VLPs in the liver and the entire organism in transgenic mice with CHB, next in CHB patients and in prospect - for **future CHB therapies**.