Immune response induced by injection-oral co-immunisation with plant-derived HBV antigens polarising the response to Th1 or Th2 type in the context of potential therapy for chronic hepatitis B

Despite massive vaccination programmes, Hepatitis B Virus (HBV) remains **the major cause of liver diseases**, mostly in developing countries. Populations there are also endangered to many other diseases of viral, bacterial and helminth origin, defined as Neglected Tropical Diseases (NTDs), which may have a substantial effect on HBV vaccination. Hence, effective treatments of **chronic hepatitis B (CHB)**, but also economically feasible and commonly available, also for patients with comorbidities, are especially desired.
Currently investigated CHB **therapeutic vaccines** and other therapies are aimed to restore HBV-specific cell-mediated response supported by the humoral one, i.e. antibody production. The main component of tested CHB therapeutic vaccines is the **HBV core antigen (HBcAg)** triggering mostly Th1 type of response - developing into cellular response, which can be supplemented with subunits of the **HBV surface antigen (HBsAg)** - primarily inducers of Th2 response - humoral one.

Plant-produced HBV antigens assembled into Virus-Like Particles (VLPs), were also used as effective vaccines. Our recent results demonstrated that plant-produced HBcAg or S-HBsAg (small HBsAg), and purified or bioencapsulated in lyophilised tissue - triggered systemic immune response with significantly increased titres of specific antibodies. The profile of IgG isotypes indicated predominantly Th1 or Th2 response for HBcAg or S-HBsAg, respectively. The results achieved separately for S-HBsAg and HBcAg, have been effective enough to **warrant further advancement**. Now, the promising results have to be supported in detailed studies to provide data on **co-immunisation using bi-antigen vaccine, containing HBcAg and S-HBsAg**.

Here, we plan a complex study on **immunogenicity of co-delivered plant-derived HBcAg and S-HBsAg, via injection priming and low-dose oral boosting regime**. We want to evaluate **mutual impact of concurrently induced Th1 and Th2 pathways on the overall immune response** in mice as model animals. We want also to investigate **if and how helminth** model (*Fasciola hepatica*) **infection affects the bi-antigen vaccine efficacy** as HBV and helminthic co-infection is a frequent scenario in developing countries. Our aim is recognition of immunisation strategy for potentially broadly applicable CHB therapy, available also for individuals with comorbidities.

We assume that **injection-oral co-immunisation** with the plant-derived HBcAg and S-HBsAg, is a **viable alternative way for inducing Th1 type of response and further T-cell mediated response, supplemented by Th2 type of response and then humoral response, both required for potential CHB therapy**. The innovative strategy is to use antigens bio-encapsulated in plant tissue to be delivered orally at boosting, instead of repetitive injections. Our **research hypothesis** is that thanks to injection priming, low-dosed orally administered antigens at boosting can interact in mesenteric lymph nodes (MLNs) - connected to Gut Associated Lymphoid Tissue, with already sensitised lymphocytes. Consequently, the systemic response is predominant whereas the mucosal response (including oral tolerance) is decreased. Then, simultaneous exposition of HBcAg and S-HBsAg will elicit response of mixed type - Th1/Th2 or cellular/humoral. We also suppose that thanks to this immunisation regime, Th1/Th2 response still can progress, even under predominant Th2 polarisation of the immune system developed as a result of parasitic infection. We plan also to test **improved HBcAg and S-HBsAg preparations**, regarding more effective plant production of the antigens and their form, i.e. of increased cell permeability, which is assumed to increase their immunogenicity. We plan to verify effect of all these factors on mutual Th1/Th2 immune response by complex analysis of **cell-mediated** (white blood cells, lymphocyte subpopulations and production of cytokines - immunomodulators) **and humoral responses (**HBc- and SHBs-specific antibodies, in serum and secretory IgA)**, both at local -** in MLNs, **and systemic** - in the blood and spleen, level on the immune response.

We believe that this basic study will provide new data on interaction between different types of antigens and exerted respective Th1 - cellular and Th2 - humoral responses. Analysis of MLNs will make possible to define reciprocal effects of parenteral and oral routes of antigens delivery on immunisation efficacy. This data can be fundamental for explanation of mechanisms of oral immunisation, particularly using plant-derived vaccines. Finally, the effect of concomitant parasitic infection will be examined regarding therapeutic vaccination. Summarising, gained knowledge on immune response elicited by the novel bi-antigen vaccine will be important for immunology, vaccinology, biopharming, CHB therapy and public health.