Recombinant virus-like particles of tick-borne encephalitis virus produced in *Leishmania tarentolae* expression system as a potential vaccine antigen

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Tick-borne encephalitis virus (TBEV) represents a significant public health concern across various regions of central, eastern, and northern Europe, as well as northern Asia, resulting in severe neurological infections, such as encephalitis or meningitis. The primary mode of transmission is through tick bites, although consumption of dairy products from infected animals such as goats, cattle, and sheep also presents a notable risk factor. Vaccines based on the inactivated virus are available against TBEV; however, no specific antiviral therapy has been developed so far. Despite the substantial increase in the number of TBEV infections, vaccination rates remain low in many affected countries due to the high costs of existing vaccines. Moreover, due to the high costs they are not widely used as veterinary vaccine, highlighting the urgent need for alternative preventive measures.

The approach presented in this dissertation based on TBEV virus-like particles (VLPs), that mimic the structure of native viral particles but lack genetic material, exhibited high potential as a vaccine antigen. Notably, a significant milestone was achieved by demonstrating the utility of using the protozoan *L. tarentolae* expression system for the efficient production of TBEV VLPs. Through functional characterization, it was confirmed that the recombinant structural proteins (prM/M and E) of TBEV formed VLPs that were highly recognized by neutralizing antibodies in *in vitro* analyses.

In order to assess the immunogenicity of the obtained particles, immunization studies were carried out in a mice animal model. The animals were vaccinated with three doses of VLPs in combination with the AddaVax™ adjuvant, and then the obtained post-immunization sera were analyzed. Analyses showed high levels of antibodies against TBEV proteins and the inactivated virus. The virus neutralization test showed a high ability to neutralize TBEV using post-immunization sera, which confirmed the effectiveness of VLPs as a vaccine antigen. The high potential of the obtained VLPs for use in a potential vaccine was also confirmed in a challenge experiment, where immunization with VLPs in combination with an adjuvant provided 100% protection against the development of symptoms of infection in mice administered with a lethal dose of the virus.

The next stage of the research was to determine the most effective route of administration and combination with an adjuvant for the potential vaccine. Building on previous findings that demonstrated the efficacy of VLP immunization in challenge experiments, the focus at this stage was to evaluate the resulting immune response profile. Using mice as an animal model, six different groups were immunized with VLPs via subcutaneous (s.c.) or intramuscular (i.m.) administration, with or without adjuvants (AddaS03™ and Alhydrogel®+MPLA). T-cell responses were analyzed using an enzyme-linked immunospot assay (ELISPOT), showing interferon γ production primarily in groups adjuvated with Alhydrogel®+MPLA.

Moreover, antibody responses were assessed, demonstrating increasing levels of antibodies after each dose, with significantly higher concentrations observed in adjuvanted groups. Additionally, IgG subclass

titers highlighted a more balanced immune response in adjuvanted groups, particularly with Alhydrogel®+MPLA in i.m. administration. Furthermore, antibody avidity analysis revealed higher avidity in adjuvanted groups, emphasizing the role of adjuvants in enhancing antibody quality. Neutralization assays demonstrated higher titers in adjuvanted groups, particularly in i.m. administration, indicating superior neutralizing potential against various TBEV strains. Overall, the findings suggest that VLPs combined with Alhydrogel® and MPLA in i.m. administration elicit a robust and balanced immune response, with enhanced neutralizing antibody production compared to previous studies. This optimized vaccination approach holds promise for effectively combating TBEV infections.

To sum up, the results obtained as part of this doctoral dissertation indicate that the obtained TBEV VLPs are a very good candidate for an alternative vaccine against this virus. Studies aimed at characterizing the produced recombinant proteins showed that they were functional and formed VLPs, which were used for further research. Their effectiveness in preventing infection has been demonstrated in several stages of animal testing. They confirmed full effectiveness in protection against infection and allowed to indicate the potentially most effective combination with an adjuvant and the route of vaccine administration.

VLPs are produced in an unconventional expression system based on a protozoan, thanks to which efficient production and purification of particles is possible, and thus a low price of the potential vaccine. This makes the developed potential vaccine competitive with currently available vaccines based on inactivated virus. The implementation of the developed recombinant vaccine with lower costs could significantly contribute to increasing the availability of the TBEV vaccine. Production costs could be reduced compared to currently available vaccines due to the use of an expression system based on cost-effective reagents, the use of simple purification methods and the recombinant antigen, which does not require such high levels of security during production as an inactivated virus-based vaccine. A cheap but effective and safe vaccine protecting against TBEV infection could be widely used. The low cost of the vaccine could also allow it to be used as a veterinary vaccine, which is not currently available. Consumption of dairy products from infected animals is one of the ways of transmitting the virus to humans. Therefore, introducing common vaccinations for farm animals would both contribute to reducing the reservoir of the virus in the environment and would also limit the possibility of human infection.