TBE NEWS

A NEW CANDIDATE TBE VACCINE BASED ON RECOMBINANTLY PRODUCED VIRUS-LIKE PARTICLES IN PROTOZOAN CELLS

Background

Five inactivated whole virus TBE vaccines adjuvanted with aluminum hydroxide based on a strain of either the Far Eastern or European subtype are currently licensed. These vaccines are highly effective and show an acceptable tolerability and safety profile. However, large quantities of pathogenic virus have to be handled during the production process, and the use of primary cells for virus replication requires special biosafety demands. Therefore, various strategies have been used to look for alternative vaccine approaches (see, e.g., Newsletter August 2020, Snapshot week 41/2018, Snapshot week 43/2018). A Polish/Czech team has now published the development of a platform by which TBE virus antigens are produced as virus-like particles (VLPs) in the protozoan *Leishmania tarentolae*. This unicellular protozoan, isolated from the Moorish gecko *Tarentola mauritanica*, is not pathogenic to mammalians and can be used under biosafety level 1 conditions.

Results

A plasmid was constructed that enables the expression of sequences of the TBE virus strain Neudoerfl, structural proteins pre-membrane protein prM and the glycoprotein E. A signal sequence from a phosphatase of *L. mexicana* was put at the N-terminus of the prM gene in order to facilitate optimal secretion of the recombinant antigen. Between the prM and E gene sequences, a three amino acid linker was inserted and a self-cleavage peptide from the teschovirus-1 virus followed by a signal sequence from TBE virus prM linked to the E protein sequence to enable its secretion. In addition, the codon usage was optimized for the production of these antigens in *L. tarentolae*.

The expression of recombinant proteins was carried out in cell cultures of recombinant protozoa using an inducible stable cell line of *L. tarentolae*. The production of the antigens was performed after tetracycline induction. Both the recombinant antigens prM/M and E were detected in cell extracts, and the recombinant proteins were also secreted in substantial amounts into the culture medium.

The recombinant viral proteins formed VLPs shown by the formation of higher density spherical particles with a diameter of approximately 50–60 nm by ultracentrifugation of the culture medium in a sucrose gradient. Treatment of the VLPs by the nonionic detergent Triton X-100 decomposed the VLPs. The efficiency of VLP production was approximately 7–10 mg per l of *L. tarentolae* culture.

Purified VLPs stored at 4°C for 18 months showed only a slight change in particle distribution which suggest that the VLPs can be stored for long periods of time. It could be shown that the two potential glycosylation sites in the prM/M and E protein were glycosylated.

The recombinant VLPs were used in immunogenicity and challenge experiments in BALB/c mice. The animals were immunized subcutaneously with three doses of 10 mg of VLPs in combination with the adjuvant AddaVax on days 0, 14 and 28. Sera of immunized mice showed high TBE antibody titers in ELISA tests and neutralization assay when the strain Hypr was used. A challenge experiment of mice with a lethal dose showed that all immunized animals did not show any symptoms of TBE 28 days post-infection, while mice in the control groups developed symptoms on day six post infection and had to be euthanized by the eleventh day.

Discussion

The recombinantly produced TBE viral VLPs showed a high immunogenic and protective potential in challenge experiments, and the efficient production of these antigens in protozoan cells were promising findings. This system may be a good candidate for cost-efficient production of effective TBE vaccines.

Literature

Zimna et al.
Functional characterization and immunogenicity of a novel vaccine candidate against tick-borne encephalitis virus based on Leishmania-derived virus-like particles
Antiviral Res. 2023;209:105511. doi.org/10.1016/j.antiviral.2022.105511

Author: Dr. Michael Bröker

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