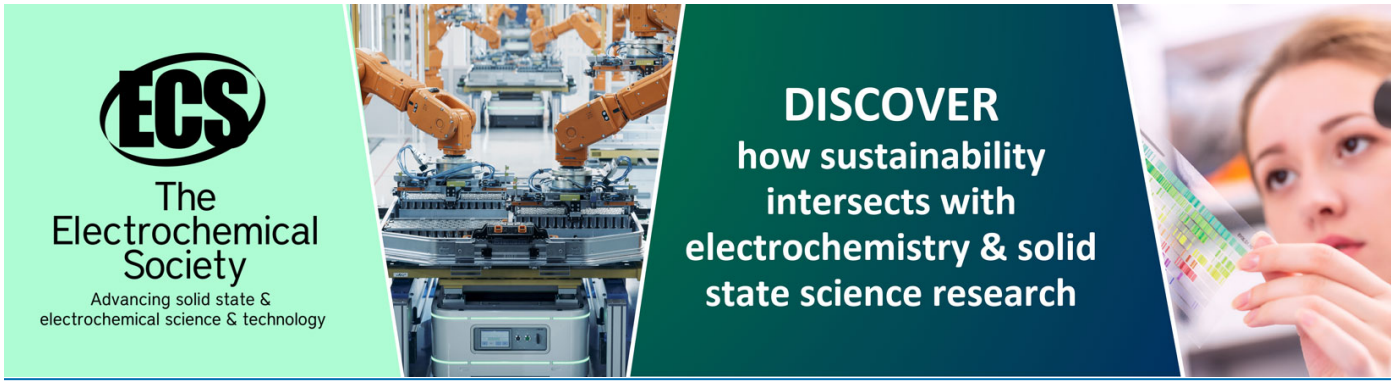
Effectivity of nanovaccine against tick-borne encephalitis



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Abstract. The tubular immunostimulating complex (TI-complex) is a new nanoparticulate antigen delivery system, which was developed to enhance immunogenicity of different subunit antigens within anti-infectious vaccines and increase their economic efficacy and safety. TI-complexes are self-organized from mixture of triterpene glycosides from *Cucumaria japonica*, cholesterol and monogalactosyldiacylglycerol (MGDG) from marine macrophytes. MGDG plays role of lipid matrix for subunit protein antigen interrupted in TI-complexes. Microviscosity of MGDG was shown to influence the conformation and immunogenicity of protein antigen. Present work was aimed to study adjuvant effect of TI-complexes on immunogenicity of chimeric protein antigen based on E protein domain III of tick-borne encephalitis (TBE) virus and OmpF porin of *Yersinia pseudotuberculosis* depending on MGDGs isolated from different marine algae and seagrass. It was shown that TI-complex including MGDG from *Ulva lactuca* was most effective. Immunization by chimeric antigen incorporated in TI-complexes provided a high level of animal protection in experimental infection with TBE. Thus, the proposed construction is promising for the development of vaccines against TBE.

1. Introduction

The search for new approaches to the prevention of infectious diseases is a priority problem of modern fundamental medicine due to the constant growth of economic damage from infectious diseases in the world. In particular, new approaches are needed to prevent such widespread and deadly viral illness as tick-borne encephalitis (TBE), since classical inactivated vaccines have various side effects and, moreover, do not provide reliable protection against a neuroviral infection. The use of safe isolated antigens of microbial pathogens in subunit vaccines allows to avoid this problem. However, most subunit antigens have insufficient immunogenicity. The obtained results showed the prospect of using nanoparticulate tubular immunostimulating complexes (TI-complexes) to deliver subunit antigens and to enhance immune response against them and microbial pathogen as a whole [1, 2]. TI-complexes are self-organized from the mixture of cholesterol with biologically active components - triterpene glycoside from marine invertebrate *Cucumaria japonica* and glycolipid monogalactosyldiacylglycerol (MGDG) from marine macrophytes (algae and seagrasses). These tubular nanoparticles are characterized by outer and inner diameters of about 16 nm and 6 nm, respectively, and the length of about 500 nm [3]. The glycolipid matrix is necessary to incorporate antigen in TI-complex. Different

physicochemical properties of MGDGs isolated from different marine macrophytes allow to influence the conformation of the incorporated protein antigen and to obtain the most immunogenic anti-infectious vaccine construction [1]. Present work was aimed to elaborate effective and safe anti-TBE vaccine construction based on TI-complex and recombinant chimeric protein, consisting of the sequence part of protein E (III domain) of TBE virus (TBEV) connected with porin OmpF of *Yersinia pseudotuberculosis* by linker sequence (OmpF-EIII) [4]. The resulting chimeric protein has a number of advantages. Thus, E protein domain III contains the main antigenic determinants of the protective antigen of TBEV. Membrane protein OmpF plays role of anchor, which provides better incorporation of the chimeric antigen in the glycolipid matrix of TI-complexes. In addition, the bacterial protein in the content of the chimeric antigen facilitates its synthesis in the expression system of *Escherihia coli*.

2. Material and methods

2.1. Animals and immunization

The mice were divided into six experimental groups (ten animals in each): (1) mice, which were injected with phosphate-buffered saline (PBS) containing 0.125% n-octylglucoside (control); (2) mice immunized with individual OmpF-EIII; (3–6) mice immunized with OmpF-EIII incorporated into TI-complexes based on MGDGs from *Ulva lactuca*, *Sargassum pallidum*, *Laminaria japonica* or *Zostera marina*, respectively. The adult BALB/c (females) mice with the body weight of 18–20 g were immunized subcutaneously twice, applying a dose of 20 μg of OmpF-EIII *per* mouse, at an interval of 14 days in all groups. Experiment was terminated 28 days after the first immunization.

2.2. ELISA

Blood sera of the experimental mice were obtained to determine the content of the anti-EIII antibodies. The blood of the mice was collected immediately after decapitation and incubated at 37 ^{o}C for 2 h. After clot retraction, the samples were centrifuged at 1500 rpm for 10 min. The supernatant was taken into plastic tubes and stored at −20 ^{o}C. The content of anti-EIII antibodies in mice blood serum was determined by ELISA as described in [5].

2.3. Protective activity against TBE

Three groups each by ten mice were immunized subcutaneously according to the procedure described above (section *2.1.*): (1) PBS containing 0.125% *n*-octylglucoside (control); mice immunized with individual OmpF-EIII; (3) mice immunized with OmpF-EIII incorporated into TI-complex. Two weeks after the second immunization, the mice were subcutaneously infected with TBEV strain Dal'negorsk [6]. Then mice were monitored daily for 21 days to survival rate.

3. Results and discussion

The adjuvant effect of TI-complexes on immunogenicity of chimeric protein OmpF-EIII [4] were evaluated by production of anti-EIII antibodies in the blood serum of mice immunized with individual chimeric antigen OmpF-EIII and OmpF-EIII incorporated in TI-complexes comprising MGDG from *S. pallidum*, *U. lactuca*, *L. japonica* or *Z. marina*. Earlier, it was shown that TI-complexes exhibit a different adjuvant effect depending on the physicochemical properties of MGDG [1]. Results of present study confirm this conclusion. So, TI-complexes containing the most viscous MGDG from *Z. marina* contributed to a decrease in the immune response in comparison with the effect of the individual chimeric antigen, while the TI-complex based on MGDG from *U. lactuca* with lower microviscosity showed the highest adjuvant activity (Figure 1).

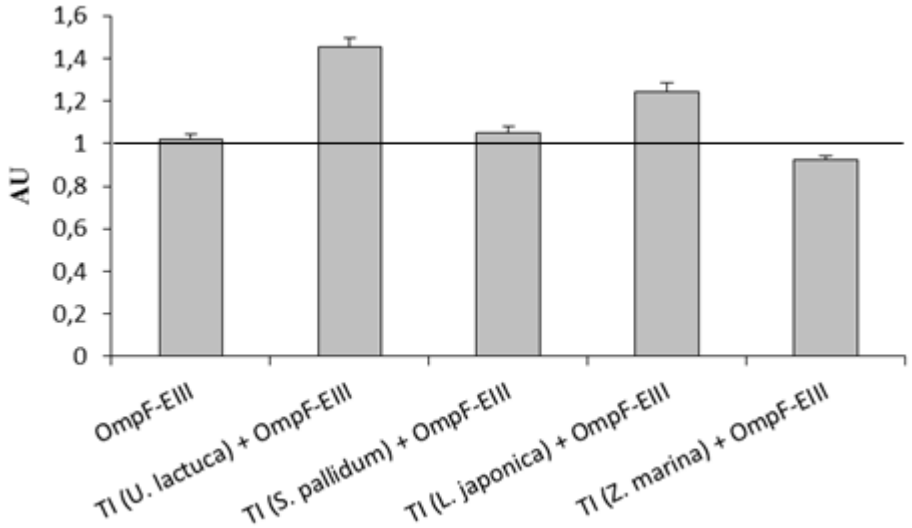

Figure 1. The content of anti-EIII antibodies in mice blood serum depending on MGDG in the composition of TI-complexes. X-axis: experimental groups of mice immunized with individual EIII-OmpF (OmpF-EIII) and OmpF-EIII incorporated in TI-complexes based on MGDG from *Ulva lactuca* (TI(U. lactuca + OmpF-EIII)), *Sargassum pallidum* (TI(S. pallidum + OmpF-EIII)), *Laminaria japonica* (TI(L. japonica + OmpF-EIII)) or *Zostera marina* (TI(Z. marina + OmpF-EIII)). Y-axis: the ratio between the content of anti-EIII antibodies in experimental groups and the control (mice injected with phosphate-buffered saline). Data are expressed in arbitrary units (AU) relative to the control value equal to 1 (the horizontal line).

A study of the protective activity of vaccine constructs based on the chimeric protein OmpF-EIII incorporated in the TI-complex showed that the mortality of mice immunized with both individual OmpF-EIII and OmpF-EIII incorporated in TI-complex was significantly reduced till 30% and 20%, respectively, against 70% in the non-immunized mice.

Hence, the chimeric protein OmpF-EIII has a high protective activity which significantly increases as a result of incorporation of the antigen into TI-complexes.

Acknowledgments
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