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# Evaluation of immune response and protective effect of four vaccines against the tick-borne encephalitis virus

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ABSTRACT

Among three main subtypes of the tick-borne encephalitis virus (TBEV), the Siberian subtype is currently dominant in a majority of the endemic regions of Russia. However, inactivated vaccines are based on TBEV strains of the heterologous Far Eastern or the European subtypes isolated 40–77 years ago. To analyze the efficacy of the available vaccines against currently prevailing TBEV isolates of the Siberian subtype, mice were immunized subcutaneously three times (one group per each vaccine). The expression of seven cytokine genes was determined using RT-PCR. Sera were studied using homologous and heterologous ELISA, hemagglutination inhibition (HI) and neutralization tests with TBEV strains of the Far Eastern, Siberian and European subtypes. Cross-protective efficacy of the vaccines was evaluated with the TBEV strain 2689 of Siberian subtype isolated from an ixodid tick from the Novosibirsk, South-Western Siberia, Russia in 2010. The cytokine gene expression profile indicates a predominantly Th2 response due to exogenous antigen presentation. Titers for homologous combinations of vaccine strain and strain in ELISA, HI and neutralization tests exceeded those for heterologous antigen-antibody pairs. Despite antibody detection by means of ELISA, HI and neutralization tests, the mouse protection afforded by the vaccines differed significantly. Complete protection of mice challenged with 100 $LD_{50}$ virus of the Siberian subtype was induced by the vaccine "Encevir" ("Microgen", Tomsk, Russia). The minimal immunization doze ($MID_{50}$) of "Encevir" protecting 50% of the mice was less than 0.0016 ml. Partial protective effect of vaccines produced in Moscow, Russia and Austria revealed $MID_{50}$ within recommended intervals (0.001–0.017 ml). However, the $MID_{50}$ for the vaccine "Encepur" (Novartis, Germany) 0.04 ml exceeded acceptable limits with total loss of mice immunized with vaccine diluted 32, 100 and 320 fold. These results suggest regular evaluation of TBEV vaccines in regions where heterologous virus subtypes prevail.



## 1. Introduction

TBEV is a causative agent of severe and potentially fatal infection of the central nervous system [1–4]. According to the International Committee for Taxonomy of Viruses, TBEV is classified as one species with three subtypes [2]: (1) the Far Eastern subtype (mainly isolates from far-eastern Russia, China and Japan); (2) the currently widely spread Siberian subtype (previously isolated from Siberia and the Urals but at present the dominant subtype in many TBEV endemic regions of Russia and surrounding countries, gradually replacing other TBEV subtypes [3]; and (3) the European subtype (which comprises almost all isolates from Europe) [1,2,4].

*Abbreviations:* TBEV, The tick-borne encephalitis virus; AFM, Atomic force microscopy; TEM, Transmission electron microscopy; RT, Reverse transcription; PCR, Polymerase chain reaction; ELISA, Enzyme-linked immunosorbent assay; PS, Porcine embryo kidney cells; HI, Hemagglutination inhibition; $TCID_{,50}$Tissue culture infection doses 50; $LD_{50}$, Lethal doses; TNF, Tumor necrosis factor; $MID_{50}$, Minimal immunization doze protecting 50% of mice; o.u., Optical units.

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Several viral isolates from Eastern Siberia, Russia and Mongolia do not belong to these main subtypes [5] (GenBank accession numbers EF469661 and EF469662, suggesting the occurrence of subtypes 4 and 5, respectively).

Immediately after discovery of TBEV in 1937, a first inactivated vaccine was developed. During 77 years of commercial exploitation, innovations and implementations of similar conventional vaccines using the same approach of formaldehyde inactivation of the virus grown in primary chick embryo fibroblasts both advantages and disadvantages became evident. All seven available vaccines against the tick-borne encephalitis produced in Russia (2), Austria (2), Germany (2) and China (1) proved their safety and efficacy in humans [1,4,6,7]. Currently, Russian vaccine strains of the Far Eastern genetic subtype include strain Sofjin isolated from a patient's brain in 1937 and strain 205 isolated from the *Ixodes persulcatus* Schulze tick in Khabarovsk region in 1973. European vaccines are based on strain Neudorfl isolated from an *Ixodes ricinus* tick in 1971 and strain K23 also originated from *I. ricinus* in 1975. In China strain Senzhang of the Far Eastern subtype isolated from a patient's brain in 1953 is used for vaccine production by the Changchun Institute of Biological Products [6]. Thus, all currently available vaccines against the tick-borne encephalitis are based on the strains heterologous to the majority of the current TBEV isolates and were isolated 40–77 years ago.

A number of countries including Russia, Austria, Germany, Finland, Hungary, Latvia, Slovenia, Switzerland and Italy possess official governmental vaccination programs [7]. In Austria 88% of the total population have a history of vaccination against TBEV and an incidence of illness below 1 per 100,000 population. By comparison in the neighboring Czech Republic vaccine coverage is 16% and the incidence of illness 7.8 per 100,000. In Slovenia the comparable figures are 12% and 13.1 per 100,000. These data are consistent with vaccine efficacy. However, Slovakia (1%, 1.3 per 100,000 population) might argue against it [1,7]. In Russia, the average vaccination rate of approximately 5% is not high enough to influence the periodic variations of incidence of the disease [8].

Inactivated vaccine failure and incomplete protection have been described [8–12]. However, the tick-borne encephalitis average rate was lower among vaccinees and fever manifestations were typical [8–11]. Thus, in the Far East of Russia in the 1990s, tick-borne encephalitis occurred in 15.1% of patients previously immunized with the inactivated vaccines. In the 2000s the incidence in vaccinees was 8.2%. The case fatality rate was 5.3 and 0%, respectively [11]. In the Republic of Altai, long-term monitoring revealed an incidence of $22.7 \pm 3.3\%$ in vaccinated patients in whom only $5.0 \pm 1.8\%$ had confirmed violations of the immunization protocol [10]. Moreover, in Russia in 2007–2011, 23.79% patients with tick-borne encephalitis had previously been vaccinated either with a complete or incomplete course. Fulminant encephalitis in 55 h with a lethal outcome was described in a patient who was vaccinated 6 times with vaccines based on the TBEV Far Eastern strains. Siberian subtype of the TBEV was isolated from the patient's spinal cord [12]. Thus, in spite of effective protection against homologous TBEV isolates, currently available vaccines do not provide complete protection against phylogenetically divergent virus isolates.

Whereas the vaccine-mediated protection against the TBEV Far Eastern and European strains is well studied [1,4,6,7,13–17], cross-reactions with the currently predominant isolates of the Siberian subtype (groups Aina-Vasilchenko [18] and Zausaev [19]) remain uncertain. Our aim was to evaluate the immune response and the protective effect of vaccines based on the Far Eastern and European subtypes against a recently isolated TBEV strain of the Siberian type.

## 2. Materials and methods

### 2.1. Virus

TBEV reference strains of the 3 main subtypes: Far Eastern (Sofjin (GenBank accession number X07755)), Siberian (Aina (AF091006) and European (Absettarov (AF091005)) were obtained from the Russian State Collection of Viruses (Ivanovsky Institute of Virology of Ministry of Health, Moscow, Russia). The TBEV strain 2689 (JQ693478) was isolated from an ixodid tick in the Novosibirsk, Russia in 2010.

### 2.2. Vaccines

Four vaccines against the tick-borne encephalitis "Encevir" (Microgen, Tomsk, Russia), IPVE (Institute of Polyomyelitis and Viral Encephalitis, Moscow, Russia), FSME-Immun (Baxter, Austria) and Encepur adult (Novartis, Germany) were examined using microscopy, immunological and molecular biology methods. The viral antigen E in the vaccines was detected by semi-quantitative

**Table 1**
Analysis of cytokine gene expression in white blood cells of mice after immunization with 4 vaccines against the tick-borne encephalitis.

| Cytokines | Percentage of mice with expression of the corresponding cytokine gene (#expressing/#tested) | | | | | Significant differences |
|---|---|---|---|---|---|---|
| | IPVE, Moscow (I) | Encevir, Tomsk (II) | FSME-Immun Austria (III) | Encepur, Germany (IV) | Control 0.9% NaCl (V) | |
| **Th1 immune response** | | | | | | |
| INF γ | 10.0 (1/10) | 50.0 (5/10) | 11.1 (1/9) | 60.0 (6/10) | 10.0 (1/10) | |
| IL12 | 20.0 (2/10) | 70.0 (7/10) | 66.7 (6/9) | 0.0 (0/10) | 22.2 (2/9) | II–IV, III–IV $p < 0.01$ |
| TNF | 40 (4/10) | 20.0 (2/10) | 22.2 (2/9) | 30.0 (3/10) | 20.0 (2/10) | |
| **Th2 immune response** | | | | | | |
| IL4 | 0.0 (0/10) | 0.0 (0/10) | 77.8 (7/9) | 0.0 (0/10) | 0.0 (0/10) | I–III, II–III, III–IV, III–V, $p < 0.01$ |
| IL6 | 0.0 (0/10) | 0.0 (0/10) | 44.4 (4/9) | 0.0 (0/10) | 0.0 (0/10) | |
| IL10 | 100.0 (10/10) | 100.0 (10/10) | 100.0 (9/9) | 80.0 (8/10) | 10.0 (1/10) | I–V, II–V, III–V, $p < 0.001$; IV–V, $p < 0.01$ |
| IL-1β | 75.0 (6/8) | 50.0 (5/10) | 66.7 (6/9) | 70.0 (7/10) | 83.3 (5/6) | |

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