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PRECLINICAL DEVELOPMENT OF A CHIMERIC YELLOW FEVER / TICK-BORNE ENCEPHALITIS VIRUS AS A CANDIDATE VACCINE

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ABSTRACT

Tick-borne encephalitis virus (TBEV) is a reemerging pathogen in Kazakhstan. Despite the availability of inactivated TBEV vaccines produced abroad, their reliance on complex multi-dose regimens and frequent boosters limits their implementation for routine use in Kazakhstan. New technologies, including chimerization of different *Flavivirus* species, enable the development of vaccine candidates which require only a single dose to achieve long-lasting immunity. The ChimeriVax platform leverages the efficient replication machinery of the yellow fever virus (YFV) 17D vaccine strain engineered to express the structural proteins of a different flavivirus.

In this work, the ChimeriVax YFV/TBEV virus was created by replacing the prM-E genes in the YFV genome with the prM-E genes of TBEV. Preclinical evaluation demonstrated robust replication (~10^8 focus-forming units, FFU/mL) in cell cultures and genetic stability over multiple passages. In murine models, the chimeric virus elicited transient viremia (peaking at 10^4 FFU/mL) without mortality even at high doses (10^5 FFU). Immunization induced potent neutralizing antibodies (geometric mean titer: 4,076) and robust cellular immunity, marked by production of the cytokines IFN-γ, TNF-α, and IL-2 upon antigen stimulation. These results position the ChimeriVax YFV/TBEV virus as a promising vaccine candidate.

Keywords: live-attenuated vaccine; Yellow fever virus; Tick-borne encephalitis virus; neutralizing antibodies; cellular immunity; preclinical development.

INTRODUCTION

Tick-borne encephalitis virus (TBEV), a medically important member of the *Orthoflavivirus* genus (Flaviviridae family), causes severe neuroinvasive disease with mortality rates ranging from 1% to 35%, depending on the viral subtype. Surviving patients often experience severe neurological sequelae [1,2]. TBEV naturally circulates in an enzootic cycle involving *Ixodes* spp. ticks and small rodents as a reservoir. The endemic area for TBEV stretches across temperate regions of Eurasia, from mainland Europe in the west, to Japan and northern China in the east. Endemic foci exist in Kazakhstan, in the country's mountain forests but also, surprisingly, in central steppe and semi-desert ecosystems [3,4]. Kazakhstan reports 30–50 annual human cases (incidence: 0,22/100000), with the Siberian subtype (TBEV-Sib) dominating local circulation [4].

Although TBEV inactivated vaccines (TBEV-IVs) are produced by European, Russian, and Chinese manufacturers and available in Kazakhstan, their complex vaccination schedule makes TBEV-IVs impractical in our country for routine vaccination campaigns [5]. Consequently, no systematic vaccination program exists for populations in endemic regions of Kazakhstan. TBEV-IVs require multiple primary doses (typically 1-3 initial shots) followed by regular booster shots every 1-2 years [5]. The need for frequent boosters stems from the relatively short duration of protective immunity, which reflects the limited immunogenicity of TBEV-IVs. This drawback has driven sustained global efforts to develop more potent TBEV vaccine platforms. Notably, such multi-dose regimens contrast sharply with single-dose live-attenuated vaccines (LAVs), such as those based on the chimeric flavivirus platform (commercialized as ChimeriVax by Sanofi) [6]. The ChimeriVax technology uses live antigens that are hybrid flaviviruses, whose genomes combine the replicative machinery of one flavivirus species (the backbone virus) with genes encoding structural proteins from another species (the envelope donor). These chimeric viruses induce production of neutralizing antibodies specific to the envelope donor virus [7]. While various flavivirus genomes have been tested as backbones, the *Yellow fever virus* (YFV) vaccine strain 17D remains the most widely used platform for ChimeriVax vaccines. The ChimeriVax technology has proven effective through the development of licensed live attenuated vaccines (LAVs) by Sanofi, including IMOJEV for *Japanese encephalitis virus* (JEV) and Dengvaxia for *Dengue virus* (DENV) [8-12]. ChimeriVax-based vaccines were first approved for mass immunization in 2010. Since then, tens of millions of people in dozens of countries have been vaccinated with either IMOJEV or Dengvaxia [8-12]. This clinical success motivates application of the platform to other medically important flaviviruses, including TBEV.

All flaviviruses share the same genome organization. Their ~11 kb RNA genome encodes structural (C, prM/M, E) and nonstructural (NS1–NS5) proteins [13]. The virus shell proteins prM-E induce neutralizing antibody (NAb) responses, which are a key correlate of protection. A chimeric flavivirus elicits an NAb response against the shell proteins donor virus, therefore the chimera can serve as a LAV against the shell donor virus. TBEV is classified into three principal subtypes: European (TBEV-Eu), Siberian (TBEV-Sib), and Far Eastern (TBEV-FE), with additional putative regional subtypes identified [14]. All TBEV subtypes share a high degree

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of sequence conservation in the envelope (E) protein, a major antigenic determinant [15]. This structural homology resulting in cross-reactive epitopes enables vaccines derived from a single subtype to elicit protective immunity against heterologous strains [16]. We developed a candidate vaccine virus ChimeriVax YFV/TBEV. The replication backbone was derived from the YFV 17D vaccine strain, while the envelope proteins were sourced from the TBEV Vs (Vs) strain, a representative of the Siberian TBEV subtype, which is the most prevalent in Kazakhstan.

MATERIALS AND METHODS

Cell Line, Virus Genomes and Molecular Cloning

BHK-21 (ATCC CCL-10) and PK-15 (ATCC CCL-33) cells were cultured in DMEM (Lonza BE12-604) supplemented with 10% fetal bovine serum (FBS; Gibco 16000-044), 2 mM L-glutamine, 1% MEM vitamins (all from ThermoScientific). Viral genes and genomes in this works were derived from *Yellow fever virus* (YFV) strain 17D (GenBank X03700) and *Tick-borne encephalitis virus* (TBEV) strain Vasilchenko (Vs) (GenBank L40361). As a starting construct for virus rescue, we used a previously described molecular infectious clone (MIC) of the yellow fever virus (YFV), which is maintained in the NCB's collection [17]. This MIC is based on the pACYC177 plasmid backbone and harbors the full-length genome of the YFV 17D vaccine strain. The TBEV prM-E genes were PCR-amplified from a cloned full-length genome of TBEV strain Vs. The MIC for the TBEV strain Vs was generated at the NCB through the assembly of synthetic DNA fragments. The live virus was subsequently rescued from this MIC (manuscript accepted for publication in *Virologica Sinica*). The ChimeriVax YFV/TBEV virus was produced by replacing the prM-E genes in the YFV genome with the prM-E genes of TBEV. All works involving live viruses were conducted in a licensed biosafety facility at the Federal State Budgetary Scientific Institution «Scientific Centre for Family Health and Human Reproduction Problems» (Irkutsk, Russia), in compliance with biosafety regulations.

Virus Rescue, Replication Kinetics and Phenotypic Stability

The live ChimeriVax YFV/TBEV virus was generated by introducing the MIC plasmid into BHK-21 cells via electroporation. The cells grown in a P150 dish to 90% confluence were electroporated with 5 µg of plasmid DNA using a Gene Pulser II (Bio-Rad) at 1,500 V, 25 µF, with 2 pulses. The electroporated cultures were incubated until the cytopathic effect (CPE) becomes evident (4–5 days post-transfection) and supernatants were harvested. Virus stocks were stored at −80°C. Replication kinetics was measured after transfection. Titer stability was measured using infectious passages. To produce a new passage, BHK-21 cells were infected with a previous-passage-virus at MOI = 0.1. Ten passages were made (P1–P10). Supernatants were collected on days 5 after infection. TBEV titration was done using plaque assay on PK-15 cells. In some experiments with chimeric viruses, viral titers were determined by focus-forming assay on PK-15 cell monolayers under an agar overlay. Following incubation, foci of dead cells were visualized by staining with the vital stain MTT (Sigma Cat. M2128; 3 mg/ml in DMEM base without additives) for 3 hours and then counted.

Animal Experiments, Viremia, Toxicology of the Vaccine Candidate

This study was approved by the Ethical Committee of the Federal State Budgetary Scientific Institution "Scientific Centre for Family Health and Human Reproduction Problems", Irkutsk, Russia (Protocol No.3.5 from 26.02.2023). Viremia was measured in CD-1 mice (6 week old, n=3 per group). On day 0, eight groups (24 mice total) were inoculated i.p. with 10^5 FFU of YFV/TBE virus in 200 µL. The placebo group (Group 9) received an equivalent volume of sterile saline and served to assess procedure-related mortality to the end of the experiment and to collect normal (baseline) sera. Every day after inoculation, one group was euthanized, and blood was collected via cardiac puncture into Vacutainer tubes without anticoagulant. Serum was separated and stored at −80°C until all samples were processed. The virus in sera was titrated by foci counting. Titers in individual animals were used to compute geometric mean titers (GMT).

Mortality after direct inoculation into brain (intracranial, i.c.) was measured using five groups of CD-1 mice (6 weeks old, n=10 per group). The mice were anesthetized using short-term diethyl ether anesthesia by placing them in a glass beaker containing ether-soaked cotton until loss of consciousness. Three groups were injected with 10^3, 10^4, and 10^5 FFU of the YFV/TBE virus in a 10 µL volume injected using a Hamilton syringe between the eye and ear in the right brain hemisphere. The placebo group received 10 µL of sterile 0.9% NaCl. An additional group was inoculated with the wild-type TBEV strain Vs (10^3 PFU). After inoculation, the mice were kept in a thermostat at 37°C until they fully recovered activity. The mice were labeled with a permanent marker to allow for daily body weight measurements. Observation continued for 21 days. Deaths in mice which could be associated with neuroinfection (accompanied with paralysis, hunched back, circling in place) were recorded daily.

Mortality after peripheral inoculation was measured using five groups of CD-1 mice (n=10). Three groups received intraperitoneal (i.p.) injections of the chimeric YFV/TBE virus at doses of 10^3, 10^4, and 10^5 FFU per mouse in a 200 µL volume, administered using an insulin syringe. Another group received saline, and yet another group was injected with the TBEV strain Vs (10^3 PFU in 200 µL). Weight changes and mortality compatible with neuroinfection were monitored for 21 days.

Neutralizing Antibody Titers, T Cell Responses

To measure the induction of TBEV-neutralizing antibodies (NAb), BALB/c mice (6–8 weeks old, one group, n=10) were used. The mice were inoculated i.p. with 10^5 FFU of the ChimeriVax YFV/TBEV virus. On day 29 post-immunization, mice were euthanized by CO_2 asphyxia, and blood was collected via cardiac puncture into anticoagulant-free tubes. Serum was heat-inactivated at 56°C for 30 min and stored at -80°C until testing in plaque reduction neutralization test (PRNT). For the PRNT, serum was diluted in phosphate-buffered saline (PBS) containing 1% FBS. The following dilutions were tested: 1:500, 1:1000, 1:2000, 1:4000, 1:8000, and 1:16000. Each dilution was mixed with 50 plaque-forming units (PFU) of TBEV and incubated for 1 hour at 37°C then transferred to 6-well plates with PK-15 cell monolayers (80-90% confluency). Upon incubation for 1 hour with cells for virus adsorption, inocula were removed and the cells were

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overlaid with agar medium. Plates were incubated at 37°C in a 5% CO_2 atmosphere for 4 days. Viral foci were counted under a microscope. Sera from non-immunized mice were used as a negative control. The NAb titers were measured in $PRNT_{50}$ units which are serum dilutions that resulted in a 50% reduction in the number of plaques compared to the virus-only control.

To assess T-cell immunity, adult BALB/c mice (8 weeks old) were inoculated i.p. with 10^5 PFU of the ChimeriVax YFV/TBEV virus or saline (placebo group). On day 21 post-immunization, mice were euthanized by CO_2 asphyxia, and spleens were aseptically harvested. Splenocytes were isolated by mechanical homogenization, filtered through a 70 µm nylon mesh, subjected to red blood cell lysis (using a buffer from Miltenyi Biotec, Cat. 130-094-183), and resuspended in IMDM medium (Sigma, Cat. I3390) supplemented with 2% FBS and 2 mM L-glutamine. For antigen-specific stimulation, UV-inactivated (254 nm, 16 h) lysates of BHK-21 cells infected with the TBEV strain Vs were added at a final concentration of 50 µg/mL. Similarly prepared lysates of uninfected BHK-21 cells served as a negative control. Splenocytes (2×10^6 cells/well) were seeded into 6-well plates and incubated for 24 h at 37°C. After incubation, conditioned media were collected and analyzed for cytokine production (IFN-γ, TNF-α, IL-2) using ELISA kits (ThermoScientific: IFN-γ, Cat. KMC4021; TNF-α, Cat. BMS607-3; IL-2, Cat. BMS601) according to the manufacturer's instructions.

Statistical Analysis

Data (e.g. PRNT) were analyzed and graphs were built using GraphPad Prism v9.0.

RESULTS

Development of the ChimeriVax YFV/TBEV Virus

To generate a live attenuated vaccine against TBEV, we engineered a recombinant viral genome in which TBEV genes prM and E coding the viral structural glycoproteins were introduced in homologous places into the backbone of the vaccine strain YFV 17D. This approach follows the established ChimeriVax strategy, where non-structural elements from the YFV 17D vaccine backbone provide replication competence, while the TBEV-derived surface antigens induce protective immunity. The ChimeriVax strategy is illustrated in Figure 1. The TBEV prM-E genes were cloned into the YFV 17D genome without any linker sequences to ensure that junctions in the viral polyprotein (C_{YFV}-prM_{TBEV} and E_{TBEV}-$NS1_{YFV}$) yield authentic viral proteins after cleavage at natural proteolytic sites. The genome of the ChimeriVax YFV/TBEV virus was cloned into a DNA-launched MIC. A DNA-launched MIC is essentially a plasmid harboring the complete viral genome under the control of eukaryotic transcription regulatory elements. In our MIC, the viral cDNA is flanked by a cytomeg-

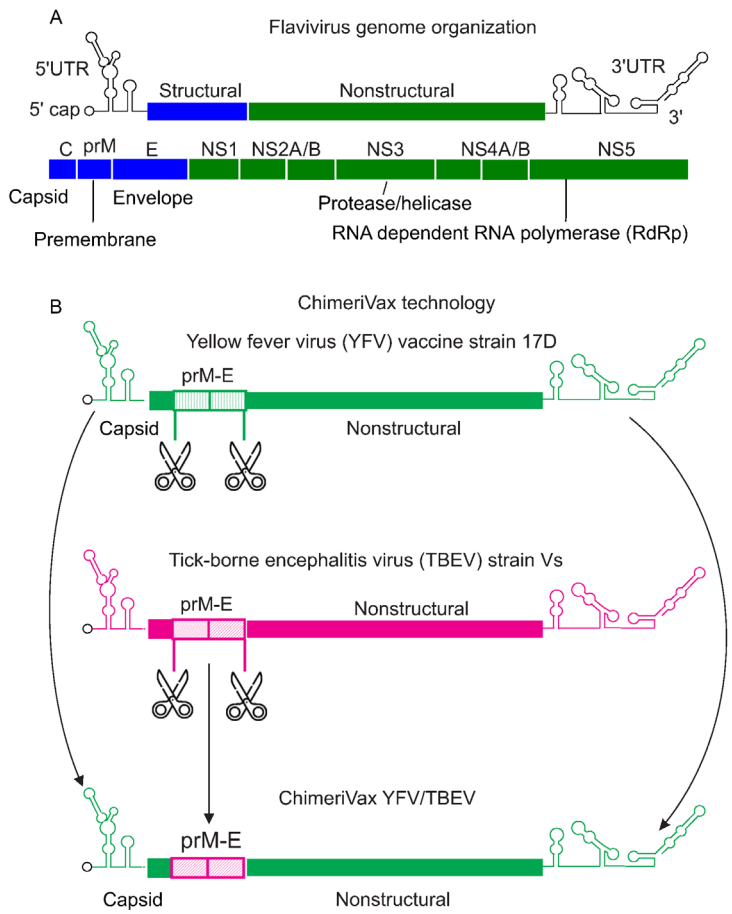

Figure 1. Organization of the flavivirus genome and principle of ChimeriVax technology. Panel A: The flavivirus RNA genome encodes three structural proteins (C, prM, E) and seven non-structural (NS) proteins (NS1–NS5). Panel B: The ChimeriVax platform is based on the attenuated *Yellow fever virus* (YFV) 17D vaccine strain. The genes coding for the prM and E structural proteins are replaced with those from a target virus, such as *Tick-borne encephalitis virus* (TBEV). The resulting chimeric virus combines the proven safety and replication efficiency of the YFV 17D backbone with the outer shell and protective antigens of the target virus, enabling the development of safe and effective live-attenuated vaccines. Source: authors.

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alovirus (CMV) immediate-early promoter at the 5' end and a hepatitis delta virus ribozyme (HDVRz) followed by a human growth hormone polyadenylation signal (hGHpolyA) at the 3' end. The resulting MIC contains the following genetic architecture of the viral insert: CMV promoter–YFV 5′UTR–C(YFV)–prM-E(TBEV)–NS1-NS5(YFV)-HDVRz-hGHpolyA. This configuration ensures accurate transcription initiation and termination of the viral RNA in transfected cells.

Viremia

As part of pharmacokinetic studies, the replication kinetics of the ChimeriVax YFV/TBEV virus were evaluated in BHK-21 cells, a cell line proposed for future manufacturing use. At 72 hours post-transfection with MIC, the virus reached a titer of approximately 10^8 infectious particles (focus-forming units, FFU)/ml (Figure 2A). Titer stability was measured by comparing the titers during ten serial passages. Despite titer fluctuations, the titer has never dropped below 6×10^7 FFU/ml demonstrating consistent viral production (Figure 2B).

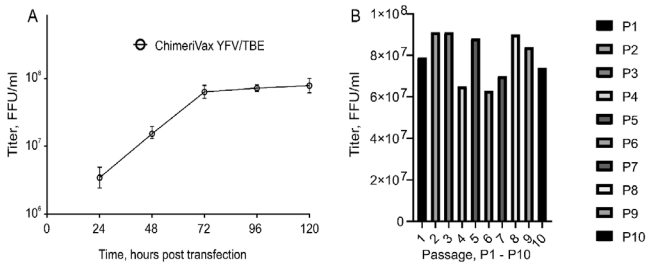

Figure 2. Replication kinetics and genetic stability of ChimeriVax YFV/TBEV. Panel A. Viral titer (FFU/ml) over time (hours post-transfection) shows peak replication around 72 hours. Panel B. Titer stability across ten serial passages (P1–P10), demonstrating consistent viral production without significant titer reduction, proving phenotypic stability. Source: authors.

Pharmacokinetic studies were extended to animal experiments by measuring viremia. Preliminary experiments demonstrated that peripheral (i.p.) infection of adult mice with the chimeric YFV/TBE virus at a dose of 10^5 FFU did not result in mortality within the 21-day observation period, which was sufficient for complete viremia resolution. Viremia in mice inoculated with the chimeric virus (10^5 FFU) was transient, persisting no longer than 5 days post-infection (Figure 3). Peak viremia was observed on day 2, reaching modest levels not exceeding 10^4 FFU/ml. No mortality occurred in any mice group. The brief viremic period and absence of mortality confirm the attenuated phenotype of the YFV/TBE virus.

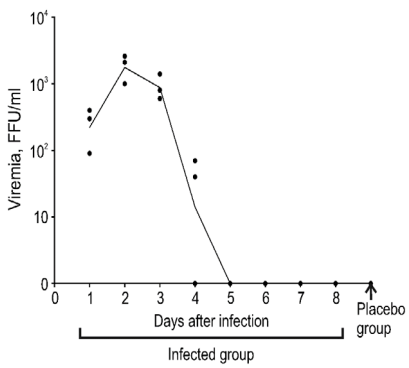

Figure 3. Viremia kinetics in mice infected with the ChimeriVax YFV/TBEV virus. Mice were inoculated with 10^5 FFU intraperitoneally. The graph depicts viral titers (FFU/ml) in sera collected at different time points after inoculation. Each dot represents an individual animal, with the curve connecting GMT. Source: authors.

Mortality Following Direct Intracranial and Peripheral Inoculation

Mortality in mice caused by the chimeric YFV/TBEV virus was compared to that caused by the wild-type TBEV (Table 1). All mice receiving the chimeric virus, regardless of the route i.c. or i.p., survived the 21-day observation period without developing overt signs of disease (ruffled fur, hunching, self-biting, impaired mobility), weight loss, or behavioral changes. In contrast, all mice inoculated with wild-type TBEV succumbed to the infection, with a median survival time of 11 days. Death was preceded by the development of neurological signs. The results in Table 1 demonstrate that the ChimeriVax YFV/TBEV virus is attenuated and is promising for further safety evaluation in different animal models.

Table 1. Mortality in infected mice

Group (dose)	Mortality (%) after i.c. inoculation	Mortality (%) after i.p. inoculation
YFV/TBE (10^3 FFU)	0	0
YFV/TBE (10^4 FFU)	0	0
YFV/TBE (10^5 FFU)	0	0
Placebo	0	0
TBEV (10^3 PFU)	10 (100%)	10 (100%)

Neutralizing Antibody after Vaccination

NAb titers were measured in mice following inoculation with 10^5 FFU of the chimeric virus. Plaque counts from the PRNT are presented in Table 2. These counts were plotted against the serum dilution (Figure 4A), dose-response curves were fitted to the data, and $PRNT_{50}$ values were calculated using nonlinear regression analysis. $PRNT_{50}$ is a serum dilution that reduces plaque counts by 50%. The $PRNT_{50}$ are shown in Figure 4B. GMT from the $PRNT_{50}$ measurements amounted to 4076.

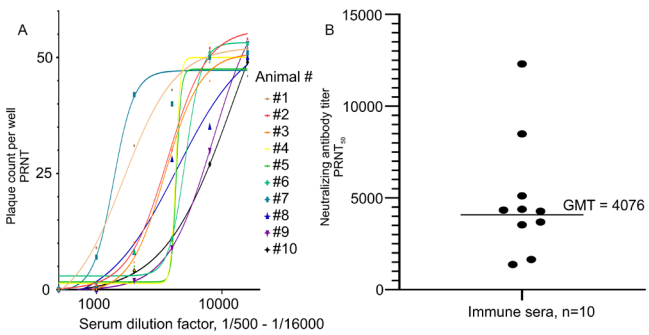

Figure 4. Neutralization of TBEV strain Vs by immune sera. Panel A. Sera were collected from mice inoculated with the ChimeriVax YFV/TBEV virus. Different serum dilutions were tested in the PRNT assay. Data points represent plaque counts per well, and the curves are nonlinear regression fits used to calculate $PRNT_{50}$ titers. Panel B. GMT for NAb titers. Source: authors.

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Table 2. Plaque counts in PRNT assay

Mouse #	Serum dilution					
	1:500	1:1000	1:2000	1:4000	1:8000	1:16000
1	0	9	31	43	51	52
2	0	1	10	30	52	54
3	0	0	6	31	45	52
4	0	0	4	10	53	47
5	0	0	5	8	49	46
6	0	1	8	11	51	53
7	0	7	42	40	50	51
8	0	0	8	28	35	50
9	0	0	2	9	30	53
10	0	0	4	11	27	49
Negative control serum (three replicates)						
1	41	46	50	54	53	55
2	43	48	54	47	50	53
3	44	49	45	51	50	47

T-Cell Immunity Responses to ChimeriVax Virus Inoculation

Antigen-specific T-cell responses were clearly observed in the group of mice immunized with the chimeric YFV/TBEV virus. Following stimulation of splenocytes with UV-inactivated lysates of TBEV-infected BHK-21 cells, a pronounced increase in T cell-produced cytokines was detected. Levels of IFN-γ ranged between 250–300 pg/mL, TNF-α reached approximately 170 pg/mL, and IL-2 was measured at around 20 pg/mL (Figure 5). In contrast, splenocytes stimulated with lysates of uninfected BHK-21 cells demonstrated cytokine levels close to the detection limit of ELISA. The differences were statistically significant ($p < 0.001$), indicating that the cytokine-production response was TBEV-specific. These data confirm the ability of the chimeric YFV/TBEV virus to induce a robust cellular immune response.

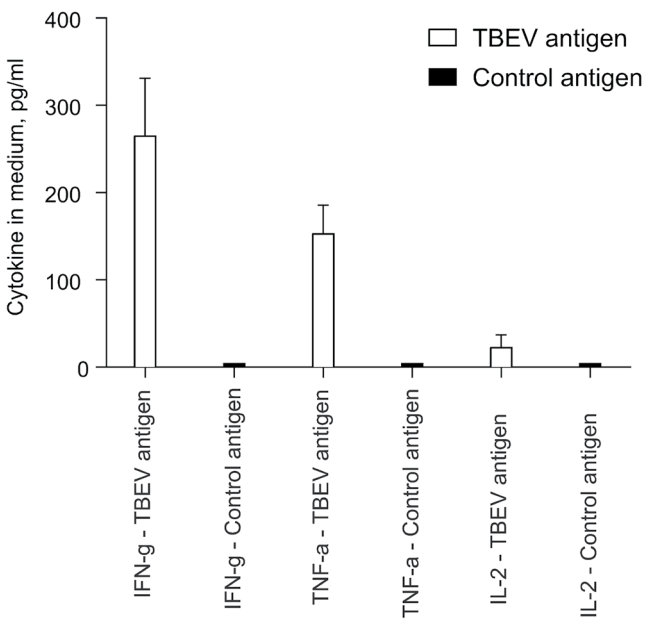

Figure 5. Cytokine production by stimulated immune splenocytes. Splenocytes from immunized mice were cultured in vitro and stimulated with the inactivated ChimeriVax YFV/TBEV antigen or control antigen without TBEV components. Cytokine levels (IFN-γ, TNF-α, IL-2) in 24-hour conditioned medium were measured in ELISA. Open bars represent mean cytokine concentrations (pg/ml) for cultures stimulated with the TBEV antigen. Black bars represent cytokines in cultures stimulated with control antigen. Source: authors.

DISCUSSION

Climate change, intensified human and animal mobility, and the expanding habitats of arthropod vectors are reshaping the epidemiological landscape of Central Eurasia with respect to vector-borne diseases. In Kazakhstan, flaviviruses transmitted by arthropod vectors - particularly TBEV and *West Nile virus* (WNV) - pose growing public health challenges. Recognizing these emerging threats, Kazakhstan has launched research initiatives to strengthen national preparedness, including the development of next-generation vaccines against endemic pathogens. Existing inactivated TBEV vaccines (TBEV-IVs) suffer from limited immunogenicity and complex immunization schedules [5]. To address these limitations, we developed a novel chimeric virus YFV/TBEV, replacing the prM-E genes of YFV vaccine backbone with those from the Siberian TBEV subtype (TBEV-Sib). The Siberian subtype predominates in Kazakhstan. This study presents the preclinical evaluation of the chimeric virus YFV/TBEV using in vitro and in vivo models.

In a recent study, Kuznetsova et al. created a YFV 17DD/TBEV chimera using the prM-E genes from the European subtype TBEV strain 493 and the replicative backbone of the YFV 17DD (derivative of 17D) strain [18]. A key methodological difference is that the authors of [18] did not develop an infectious clone. Instead, they employed the infectious subgenomic amplicon (ISA) technique. The ISA method involves transfecting cells with a mixture of several long, overlapping PCR amplicons that together constitute the entire viral genome. Homologous recombination between the ends of the PCR amplicons can occur within transfected cells, leading

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to the *in vivo* assembly of the complete viral genome and the rescue of infectious chimeric virus. Regarding the complexity of genetic engineering, the ISA method is technically simpler and less labor-intensive, because the assembly of full-length flavivirus genomes poses cloning difficulties [20-24]. However, as the *in vivo* recombination is a stochastic process and can introduce mutations, it may result in a heterogeneous viral population. In our study, we intentionally chose technically more challenging assembly of a DNA-launched MIC which contains the complete viral genome. This traditional method avoids the reliance on *in vivo* recombination and reduces risks of unintended mutations in the rescued virus.

Our chimeric virus demonstrated efficient growth in BHK-21 cells, reaching titers of ~10^8 FFU/ml and maintained genetic stability over ten serial passages. The relatively high titers and genetic stability are attributed to the efficient replication and the genetic stability inherent to the YFV 17D vaccine backbone [25]. However, *in vivo* replication was limited, as the virus in the blood of infected mice reached a maximum of ~10^4 FFU/ml and was completely cleared by day 5. With regard to safety, our ChimeriVax YFV/TBEV virus caused no mortality in murine models even after direct brain inoculation with 10^5 FFU. In the same experiments, the wild-type TBEV caused 100% mortality at lower doses 10^3 PFU. One previously reported similar chimera (YFV 17DD/TBEV) at a high dose (10^7 PFU) caused lethal encephalitis in mice [18]. We attribute the difference in virulence between our study and the reported study to the fact that our chimeric virus contains prM-E genes derived from a different strain (Vs, Siberian subtype). In this context, the higher replication efficiency of our chimera in cell culture (~10^8 FFU/ml) compared to the chimera described by Kuznetsova et al. [18] (~10^6 FFU/ml) may indicate a greater degree of adaptation to *in vitro* conditions. This type of adaptation, often referred to as heparan sulfate-dependent adaptation, is frequently accompanied by reduced replication fitness and pathogenicity *in vivo*, as has been described for many flaviviruses [26-30]. Overall, the safety characteristics observed in this study are consistent with the well-established safety profile of the YFV 17D vaccine [31], as well as with the documented records of approved chimeric vaccines against JEV and DENV [8–12].

Immunogenicity studies revealed that a single dose of our chimeric virus elicited potent NAb responses, with GMT reaching 4076, substantially exceeding the putative protective threshold ($PRNT_{50}$ >10) established in humans [19]. The chimeric YFV 17DD/TBEV virus developed by Kuznetsova et al. [18] induced only low neutralizing antibody titers, as a dose of 10^5 PFU elicited a maximum titer of 1:40 (measured as NT_{90}, the dilution required for 90% neutralization) against the TBEV strain Absettarov (European subtype). Despite direct comparisons of titers across studies are complicated by variations in assay protocols, e.g. specific virus strains, the key finding in our study is the demonstrable capacity of a single dose to elicit a potent humoral immune response.

In addition to strong humoral immunity, our chimeric virus induced robust T cell responses, as indicated by elevated production of IFN-γ, TNF-α, and IL-2 by immune splenocytes. However, it is important to note that the robust humoral and cellular immune responses reported here were measured within a short-term observational window. Therefore, studies conducted over extended time periods are required to confirm the durability of the immune response.

CONCLUSION

This study presents data on a preclinical evaluation of a chimeric flavivirus which utilizes the replicative machinery of yellow fever virus and envelope protein genes from tick-borne encephalitis virus. The characteristics of the chimeric virus demonstrate its strong potential as a vaccine candidate. The virus achieved high titers of 10^8 FFU/ml in cell culture, which is robust for flaviviruses, while in vivo it induced only a low-level (10^4 FFU/mL) and transient viremia. Furthermore, the chimeric virus caused no mortality in 6-week-old mice even after direct intracerebral inoculation at a dose of 10^5 FFU, confirming its attenuated phenotype.

A key outcome is the high immunogenicity of the candidate vaccine. A single administration elicited a potent humoral immune response, with a geometric mean titer (GMT) of neutralizing antibodies reaching 4076. In addition, a robust cell-mediated response was induced, as evidenced by a significant increase in the production of key cytokines (IFN-γ: 250–300 pg/mL, TNF-α: ~170 pg/mL, IL-2: ~20 pg/mL) by antigen-stimulated splenocytes from immunized mice.

The obtained data, including quantitative measures of replication, stability, safety, and immunogenicity, justify advancing to further preclinical studies in models more relevant to humans. The successful development of a chimeric flavivirus and the use of infectious clone technology mark significant progress in building national capacity in molecular virology. The ChimeriVax technology holds promise for creating a safe, immunogenic, and scalable single-dose vaccine against tick-borne encephalitis.

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ДОКЛИНИЧЕСКАЯ РАЗРАБОТКА ХИМЕРНОГО ВИРУСА ЖЕЛТАЯ ЛИХОРАДКА / КЛЕЩЕВОЙ ЭНЦЕФАЛИТ В КАЧЕТВЕ КАНДИДАТНОЙ ВАКЦИНЫ

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АННОТАЦИЯ

Вирус клещевого энцефалита (TBEV) присутствует в Казахстане и является вновь-возникающим патогеном. Несмотря на возможность применения инактивированных вакцин против ВКЭ зарубежного производства, в Казахстане нет массовой вакцинации населения в очах TBEV. Одной из причин является сложность протоколов вакцинации, с регулярной ревакцинацией для поддержания защитного уровня иммунитета. Новые технологии, такие как конструирование химерных вирусов из генов разных видов флавивирусов, позволяют разрабатывать кандидатные вакцины, которые требуют введения только одной дозы для получения длительной защиты. Например, технология ChimeriVax использует репликативный аппарат аттенуированного (вакцинного) штамма 17D вируса жёлтой лихорадки (YFV) для экспрессии структурных белков других вирусов из рода флавивирусов.

В данной работе химерный вирус ChimeriVax YFV/TBEV был создан путём замены генов prM-E в геноме вируса YFV на гены prM-E TBEV. Доклинические исследования продемонстрировали активную репликацию (~10^8 фокусообразующих единиц, FFU/мл) в клеточных культурах и генетическую стабильность при множественных пассажах. На мышиных моделях химерный вирус вызывал краткую виремию (пик до 10^4 FFU/мл) без летальности даже при высоких дозах (10^5 FFU). Иммунизация индуцировала нейтрализующие антитела (средний геометрический титр: 4076) и клеточный иммунитет, который был охарактеризован по продукции цитокинов IFN-γ, TNF-α и IL-2 в ответ на антигенную стимуляцию. Эти результаты позиционируют вирус ChimeriVax YFV/TBEV в качестве перспективного вируса для кандидатной вакцины.

Ключевые слова: живая аттенуированная вакцина; вирус жёлтой лихорадки; вирус клещевого энцефалита; нейтрализующие антитела; клеточный иммунитет; доклиническая разработка.

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КЕНЕ ЭНЦЕФАЛИТІ / САРЫ БЕЗГЕК ХИМЕРЛІК ВИРУСЫНЫҢ КАНДИДАТТЫ ВАКЦИНА НЕГІЗІНДЕГІ КЛИНИКАҒА ДЕЙІҢГІ ЗЕРТТЕУІ

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АҢДАТПА

Кене энцефалитінің вирусы (TBEV) Қазақстанда бар және жаңадан пайда болатын патоген болып табылады. Шетелдік өндірістің ДСЭ қарсы белсенділігі жойылған вакциналарды қолдану мүмкіндігіне қарамастан, Қазақстанда TBEV көзінде халықты жаппай вакциналау жоқ. Себептердің бірі иммунитеттің қорғаныш деңгейін ұстап тұру үшін үнемі ревакцинациямен вакцинациялау хаттамаларының күрделілігі болып табылады. Флавивирустардың әртүрлі түрлерінің гендерінен химерлік вирустарды құрастыру сияқты жаңа технологиялар кандидаттық вакциналарды әзірлеуге мүмкіндік береді, олар ұзақ қорғауды алу үшін тек бір ғана дозаны енгізуді талап етеді. Мысалы, ChimeriVax технологиясы флавивирус тектес басқа вирустардың құрылымдық ақуыздарын экспрессиялау үшін сары қызба (YFV) вирусы 17D аттенуацияланған (вакциналық) штаммның репликативті аппаратын пайдаланады.

Бұл жұмыста химерлік ChimeriVax YFV/TBEV вирусы YFV вирусының геномындағы prM-E гендерін prM-E TBEV гендеріне ауыстыру жолымен құрылды. Клиникаға дейінгі зерттеулер жасушалық өсірінділерде белсенді репликацияны (~ 10^8 фокус жасаушы бірліктер, FFU/мл) және көптеген жолақтар кезінде генетикалық тұрақтылықты көрсетті. Тышқан үлгілерінде химерлік вирус тіпті жоғары дозада (10^5 FFU) өлім-жітімсіз қысқа виремияны (шыңы 10^4 FFU/мл дейін) тудырды. Иммундау бейтараптандырушы антиденелерді (орташа геометриялық титр: 4076) және антигендік стимулацияға жауап ретінде IFN- γ, TNF- α және IL-2 цитокиндер өнімдері бойынша сипатталған жасушалық иммунитетті индукциялады. Бұл нәтижелер кандидаттық вакцина үшін перспективалы вирус ретінде ChimeriVax YFV/TBEV вирусын көрсетеді.

Түйін сөздер: тірі аттенуирленген вакцина; сары безгек вирусы; кене энцефалитінің вирусы; бейтараптандыратын антиденелер; жасушалық иммунитет; клиникаға дейінгі әзірлеу.