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The host cell response to tick-borne encephalitis virus

Tea Carletti, Mohammad Khalid Zakaria, Alessandro Marcello*

Laboratory of Molecular Virology, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

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ABSTRACT

Tick-borne encephalitis virus is the most prevalent autochthonous arbovirus in Europe and an important travel-associated virus. Complications of the infection could lead to lethal encephalitis in susceptible individuals. However, despite its clinical relevance and expanding geographical distribution, most of our knowledge on its pathogenesis is inferred from studies on other flaviviruses. Molecular details of the host cell response to infection are scarce leading to a poor understanding of the antiviral pathways and viral countermeasures that are critical to determine the outcome of the infection. In this work the relevant literature is reviewed and the key elements of tick-borne encephalitis virus infection of human cells are identified, which requires further investigation.

1. Introduction

Flaviviridae is a large family of enveloped RNA viruses, which share similarities in virion morphology, genome organization and replication strategies. The genus *Flavivirus* consists of more than 70 viruses that are transmitted to humans by arthropod vectors. Members of this genus include widespread human pathogens delivered by mosquitoes such as Dengue virus (DENV), Zika virus (ZIKV), Yellow fever virus (YFV), West Nile virus (WNV) and Japanese Encephalitis virus (JEV). Tick-borne encephalitis virus (TBEV) is the most prominent member of the TBEV complex, which includes antigenically related viruses including Omsk haemorrhagic fever virus (Siberia), Kyasanur Forest disease virus (India), Akhrma virus (Saudi Arabia), Louping ill virus (UK), Powassan virus (United States and Russia) and Langat virus (Malaysia). TBEV includes three sub-types, namely Far Eastern, Siberian and Western European. TBEV is transmitted by ticks of the species *Ixodes ricinus* (Western TBEV) or *Ixodes persulcatus* (Eastern/Siberian TBEV). Maximum incidence of human infections coincides with seasonal peaks of feeding activity of the ticks, usually in spring. The sylvatic cycle is sustained by small mammals in the forest, which do not generally succumb to the infection [1,2]. Humans are occasional dead-end hosts, who become infected by a tick bite or by consumption of raw milk from infected domestic animals. Approximately 5000–12,000 cases of TBE are reported in Europe each year [3]. The incubation period of TBEV is between 7 and 14 days with generally mild symptoms that include fever, fatigue, pain and headache. In some patients the infection causes damage to the central nervous system, which could be fatal particularly in elderly people. As observed in 20–30% of cases, encephalitis caused by European TBEV is biphasic with fever during the first phase and neurological disorders during the second phase. In contrast with severe Eastern subtype virus infection symptoms are usually milder, with case fatality rates as low as 1–2%, mostly without *sequelae*. TBEV tends to occur focally even within endemic areas. Currently, the highest incidences of clinical cases are being observed in the Baltic States, Russian Federation and Slovenia. However, autochthonous cases are constantly being reported in new areas of Western Europe showing an expansion to non-endemic areas [4]. A protective vaccine derived from inactivated Western TBEV is available and its efficacy is demonstrated by the lower prevalence of TBEV infection in highly endemic Austria, which implemented a program of vaccination with a high coverage of the population [5,6]. No drugs are licensed for TBEV, although some compounds have been tested [7].

The focus of this review is on the host cell response to TBEV infection. The transmission cycle of TBEV between ticks, vertebrate reservoirs and humans is analysed to gain information on the cellular targets *in vivo*. Also, the interferon response to TBEV

* Corresponding author. Laboratory of Molecular Virology, International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano, 99, 34149 Trieste, Italy.
E-mail address: marcello@icgeb.org (A. Marcello).
URL: http://www.icgeb.org/molecular-virology.html

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infection is considered together with the TBEV escape strategies identified so far. Finally, the integrated stress response to TBEV infection and its antiviral role is discussed. The final picture will instruct on the key steps to be implemented in the future research on TBEV infection.

1.1. General features of the virus

Mature virions are about 50 nm in diameter and are composed of an electron dense core surrounded by a lipid bilayer containing two envelope glycoproteins, E (envelope) and M (membrane). Capsid (C) protein and a positive sense single stranded RNA of 11kb make up the viral core. The genome encodes a single long open reading frame (ORF) flanked by a 5′ and 3′ noncoding regions (NCR). The ORF is translated into a polyprotein of about 3400 amino acids, which is cleaved into the viral proteins by host and viral (NS2B/NS3) proteases. The structural proteins capsid (C), pre-membrane (prM) and envelope (E) precede the nonstructural proteins NS1, NS2A, NS2B, NS3 (helicase and protease), NS4A, NS4B and NS5 (RNA-dependent RNA polymerase RdRp and methyltransferase). The 5′ NCR lacks sequence conservation, but secondary structures in this region are conserved among different Flaviviruses, albeit with some differences between mosquito and Tick-borne viruses. These structures are functionally important as *cis*-acting regulatory elements for genome cyclization, minus-strand synthesis and translation [8,9]. The 3′-NCR of TBEV is extremely variable in length, ranging from about 450 to 800 nucleotides in natural isolates [10]. It is further subdivided into a highly conserved “core” region of about 340 nucleotides at the distal 3′-end and a “variable” region between the core and the end of NS5. The core consists primarily of conserved RNA secondary structures required for cyclization that are essential for viral replication [11,12]. The variable region lacks sequence conservation and can be of different lengths. In some TBEV isolates the variable region contains an internal poly(A) tract of consecutive adenine residues [11,13]. The Neudoerfl strain contains between 30 and 250 adenosine stretches, while the highly virulent Hypr strain counts only few nucleotides [10]. While it has been shown that the components of the variable region are not essential for virus growth in cell cultures, it still remains to be established whether they might have an effect on viral pathogenesis. TBEV genomes sequenced directly from engorged ticks to avoid laboratory adaptation demonstrated that a pool of TBEV quasispecies exist in ticks, which shifts when the virus switches between invertebrate and vertebrate environments [14]. Moreover, an abundant 0.3–0.5 kb non-coding RNA fragment (termed sfRNA for subgenomic flaviviral RNA) has been detected in cells infected by Flaviviruses including tick-borne members [15]. The sfRNA is derived from incomplete degradation of the viral 3′ NCR by the cellular 5′-3′ exonuclease Xrn1 that stops at specific stem-loop structures (SL2I and SL1 in TBEV) found in the 3′-NCR. It has been demonstrated that the sfRNA regulates multiple cellular pathways to facilitate flaviviral pathogenicity and to inhibit the interferon/stress response [16,17]. Intriguingly, the sfRNA of Flaviviruses deploys RNA interference (RNAi) suppressor activities in arthropod cells [18,19].

1.2. TBEV entry and dissemination routes

TBEV enzootic transmission cycles are determined by the interaction between viruses, ticks, and their vertebrate hosts [20,21]. Vertical trans-ovarian transmission of TBEV, from an infected adult female tick to its offspring, as well as horizontal transmission to ticks *via* feeding on an infected vertebrate host has been well documented. However, the most important route of transmission for TBEV in the wild is believed to be non-viremic transmission by co-feeding ticks [22,23]. Ticks feed in clumps on hosts and simultaneous feeding of infected and uninfected ticks (co-feeding) on the vertebrate host is the pre-requisite for transmission. Skin explants of feeding sites contain migratory dendritic cells (DC) and neutrophils containing viral antigen. Moreover, migratory monocyte/macrophages were shown to produce infectious virus. Therefore, cellular infiltration of tick feeding sites and their migration between sites provides a vehicle for transmission between co-feeding ticks [24]. Intriguingly, the saliva of feeding ticks has been shown to enhance this mode of transmission [22]. During the blood meal, 33–50% of the fluid ingested by the tick is excreted back into the host [25]. Thus, tick feeding involves alternation of blood ingestion and saliva secretion for protracted periods of up to 2 weeks or more.

Transmission of TBEV to humans generally occurs following the bite of an infected tick. Ticks remain attached for long periods of time until detected and removed, which is very different from what happens following a mosquito bite. Another documented route of human TBEV infection is associated with the consumption of raw milk, usually from infected goats. The human digestive tract was shown to be an efficient route of infection, which was confirmed in early experiments with mice fed orally with TBEV [26]. Laboratory TBEV infections linked to accidental needle-stick injuries or aerosol infections have also been documented, highlighting the need of implementing accurate safety procedures [27].

Upon inoculation of TBEV into the human skin, initial infection and replication occurs in local DCs, macrophages and neutrophils causing primary viremia [24]. DCs are believed to transport virus to nearby lymph nodes, which is followed by the development of secondary viremia. This picture is mostly inferred from studies on other Flaviviruses since it has not been explored much in the case of TBEV. During the secondary viremic phase, the virus crosses the blood-brain barrier (BBB) and enters the brain [28]. Major hallmarks of TBEV neuropathogenesis are neuroinflammation, followed by neuronal death and disruption of the blood-brain barrier [29]. Neuronal injury may be directly caused by viral infection, but destruction has also been attributed to infiltrating immunocompetent cells (mainly $CD8^+$ T-cells), inflammatory cytokines and activated microglial cells [30]. TBEV infects and replicates in neurons *in vitro* inducing membrane rearrangements typical of TBEV replication and autophagosome [31–33].

1.3. The intracellular TBEV life cycle

Flavivirus TBEV particles are enveloped in an icosahedral cage of protein E dimers that completely cover the membrane and mediate both receptor binding and membrane fusion [34]. The atomic structure of the TBEV E protein in both its pre- and post-fusion conformation has been resolved as well as the conformational changes that lead to membrane fusion in acidic endosomes [35,36]. However, the uncoating of the nucleocapsid containing the viral protein C and the genomic RNA into the cytosol followed by a Cap-dependent first round of translation of viral RNA is poorly characterized. The multi-transmembrane domain polyprotein precursor localized on the endoplasmic reticulum (ER) is then co- and post-translationally cleaved by cellular enzymes (signalase and Furin) and by the viral NS2B-NS3 protease into the three structural and seven non-structural viral proteins. After translation of the genomic input RNA, the NS5 RdRP synthesizes a genome length minus strand RNA, which then serves as a template for the asymmetric synthesis of additional plus strand RNA. The newly synthesized positive strand RNA can be subsequently used for several purposes: for further translation of viral proteins, for synthesis of additional negative strand RNA, or to be incorporated into new viral particles. Hence, the viral RNA genome has three different functions:

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