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Original article

Properties of the tick-borne encephalitis virus population during persistent infection of ixodid ticks and tick cell lines

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ABSTRACT

Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis (TBE), a vector-borne zoonotic neuroinfection. For successful circulation in natural foci the virus has to survive in the vector for a long period of time. Information about the effect of long-term infection of ticks on properties of the viral population is of great importance. In recent years, changes in the eco-epidemiology of TBEV due to changes in distribution of ixodid ticks have been observed. These changes in TBEV-endemic areas could result in a shift of the main tick vector species, which in turn may lead to changes in properties of the virus.

In the present study we evaluated the selective pressure on the TBEV population during persistent infection of various species of ticks and tick cell lines. TBEV effectively replicated and formed persistent infection in ticks and tick cell lines of the vector species (*Ixodes* spp.), potential vectors (*Dermacentor* spp.) and non-vector ticks (*Hyalomma* spp.). During TBEV persistence in *Ixodes* and *Dermacentor* ticks, properties of the viral population remained virtually unchanged. In contrast, persistent TBEV infection of tick cell lines from both vector and non-vector ticks favoured selection of viral variants with low neuroinvasiveness for laboratory mice and substitutions in the E protein that increased local positive charge of the virion. Thus, selective pressure on viral population may differ in ticks and tick cell lines during persistent infection. Nevertheless, virus variants with properties of the original strain adapted to mouse CNS were not eliminated from the viral population during long-term persistence of TBEV in ticks and tick cell lines.

1. Introduction

Tick-borne encephalitis virus (TBEV) is a causative agent of neuroinfection with severe consequences in humans, tick-borne encephalitis (TBE), and is endemic in a large part of Eurasia. TBEV has been detected in many ixodid tick species (Labuda and Nuttall, 2004), but *Ixodes ricinus* and *Ixodes persulcatus* are the main vectors. In some TBEV-endemic areas, where *I. ricinus* and *I. persulcatus* ticks are absent or few in number, other ixodid tick species play the main role as TBEV vectors: *Haemaphysalis* spp. in the Far East of the Russian Federation and in Korea (Hoogstraal, 1966; Ko et al., 2010), and *Ixodes ovatus* in Japan (Takashima, 1998).

TBEV is an enveloped, unsegmented positive-stranded RNA virus. Its virion consists of a nucleocapsid with viral RNA in complex with the C protein and a lipid envelope with viral proteins M and E on its surface. E protein plays a major role in infection by mediating cell receptor binding and membrane fusion, and is also the main target for neutralising antibodies. The viral RNA is nearly 11 kb in length and has a single open reading frame, encoding a polyprotein which is processed into three structural (C, (pr)M, E) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins. This open reading frame is flanked by 5‘ and 3‘ non-translated regions (NTR) (Lindenbach et al., 2013).

There are three TBEV subtypes: European, Siberian and Far-Eastern

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(King et al., 2012). The main vector of the European subtype is *I. ricinus*, and that of the other subtypes is *I. persulcatus*. In central and north-western parts of Russia and in eastern Estonia and Latvia, which are known to be areas sympatric for the above-mentioned tick species, the Siberian subtype can be isolated from *I. ricinus* ticks and the European subtype from *I. persulcatus* (Demina et al., 2010; Katargina et al., 2013). Currently, changes in habitat and climate have led to a change in the distribution of ticks (Bugmyrin et al., 2013; Jaenson et al., 2016; Tokarevich et al., 2011) and as a consequence to the emergence of new TBE foci and to switching of the vector. Thus, in Finland new TBE foci were described where unusual combinations of *I. ricinus/I. persulcatus* ticks and European and Siberian TBEV subtypes were present with no sign of sympatry of ticks or virus subtypes (Jääskeläinen et al., 2006, 2016).

Due to the changing TBE epidemiology, the question of the properties that determine the competence of ticks as TBEV vectors becomes relevant. The role of *Dermacentor* ticks in maintaining virus circulation in natural foci has been actively discussed (Wójcik-Fatla et al., 2011; Belova et al., 2013a, 2013b; Kahl and Dautel, 2013; Karbowiak et al., 2015; Földvári et al., 2016). There is evidence indicating the ability of *Dermacentor* ticks to harbour virus for a long time (Nosek and Kozuch, 1985), and to transmit it through co-feeding (Jones et al., 1987; Alekseev and Chunikhin, 1991), transstadially (Karbowiak et al., 2016) and transovarially (Danielová et al., 2002; Zhmaeva and Pchelkina, 1967). Nevertheless, an independent TBEV vector must not only be capable of harbouring and transmitting virus both horizontally and vertically, but must also be able to maintain TBEV circulation in nature independently. Therefore, until confirmatory evidence becomes available, *Dermacentor* ticks can only be considered as potential TBEV vectors.

Besides biological and ecological characteristics of the vector, the ability of TBEV to adapt to and replicate in both the vertebrate host and the tick is an important factor for successful virus circulation in the natural focus. The level of virus replication in ticks can be one of the main factors that determine vector competence. It has been shown that the European subtype of TBEV replicates more intensively and reaches 10–100 times higher titres in tick cell lines from the main virus vector (*I. ricinus*), than in cell lines from non-vector ticks (*Hyalomma anatolicum, Rhipicephalus [Boophilus] microplus, Rhipicephalus appendiculatus* and *Ornithodoros moubata*) (Růzek et al., 2008b).

Change of the main vector may cause changes in virus population properties. TBEV variant M, adapted to *Hyalomma marginatum* ticks, reached higher titres in *Hyalomma turanicum* ticks than the parental strain EK-328, and maintained its properties during 5 successive passages through mouse brains (Dzhivanian et al., 1988; Romanova et al., 2007). Variant M also differed from the parental strain in displaying low neuroinvasiveness (ability of the virus to enter the central nervous system (CNS) and cause disease) for laboratory mice, and small-plaque phenotype in PEK cells. Variant M differed from the parental strain EK-328 genome in 15 nucleotide and 6 amino acid substitutions (Romanova et al., 2007). Moreover, the amino acid substitution in the E protein of $Glu_{122} \rightarrow Gly$, increasing the local positive charge (lpc) of the virion, was shown to be responsible for the respective phenotypes of the viral populations.

Most likely, TBEV variants with low neuroinvasiveness for mice and lpc-substitutions in the E protein have an advantage during virus replication in *I. ricinus* ticks (Labuda et al., 1994; Khasnatinov et al., 2009). It is likely that substitutions in the other genome regions could also be connected with neuroinvasiveness of the virus (Růzek et al., 2008a).

In active natural TBE foci, virus is continually transferred from the vector to the host and vice versa. In situations with a small number of hosts and unfavourable climatic conditions, virus may be able to survive for a long time in the natural focus due to persistent infection of the vector or the vertebrate host (Bakhvalova et al., 2006).

In the present study, using usual and unusual vector ticks and cell lines derived from unusual and non-vector tick species, we analysed the effect of persistent infection with the Siberian subtype of TBEV on genetic and phenotypic properties of the viral population.

2. Materials and methods

2.1. Cells

A pig embryo kidney (PEK) cell line (Institute collection, originally obtained from Mechnikov Moscow Research Institute of vaccines and sera, 1959–1965) was maintained at 37° C in Medium 199 (PIPVE, Russia), supplemented with 5% foetal bovine serum (FBS, Gibco) (Romanova et al., 2007).

In the present investigation we used cell lines derived from embryos of the ticks *Hyalomma anatolicum* – HAE/CTVM8 (Bell-Sakyi, 1991) and *Ixodes ricinus* – IRE/CTVM19 (Bell-Sakyi et al., 2007) provided by the Tick Cell Biobank, then at The Pirbright Institute. The tick cell lines were maintained at, respectively, 32° C and 28° C as described earlier (Bell-Sakyi, 1991; Weisheit et al., 2015).

2.2. Ticks

In all experiments we used first generation laboratory tick colonies (adult ticks derived from eggs laid under laboratory conditions by female ticks collected from the field) of the following species:

- *I. ricinus* (parental females from Kaluga region, Russia);
- *I. persulcatus* (parental females from the Republic of Karelia, Russia);
- *Dermacentor reticulatus* (parental females from Kaluga region, Russia)

All parental females were analysed after oviposition for contamination with TBEV, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis/E. muris* using the commercial kit AmpliSens® "TBEV, B. burgdorferi s.l., A. phagocytophilum, E. chaffeensis/E. muris-FRT" (FBIS Central Research Institute of Epidemiology, Russia) and found to be negative. Live ticks were kept at room temperature in humidified glass tubes (Belova et al., 2012).

2.3. Viruses

In our work we used

- strain EK-328, Siberian subtype of TBEV, isolated from a pool of *I. persulcatus* ticks in 1972 in Estonia; differs from the GenBank sequence (GenBank ID DQ486861.1; Romanova et al., 2007) by 2 mutations in nucleotide positions 483 and 9888, and by 2 positions (2965 and 5241) with minor heterogeneity. All substitutions were synonymous and did not affect strain properties. Passage history: 10 passages through mouse brains, 1 passage in PEK cells.
- Variant M obtained after strain EK-328 adaptation to *H. marginatum* ticks (Chunikhin and Dzhivanyan, 1979; Romanova et al., 2007). Passage history: 3 passages through mouse brains of the strain EK-328 (original isolate from the pool of *I. persulcatus* ticks), 11 parenteral passages in *H. marginatum* ticks, 1 cloning in PEK cells, 6 parenteral passages in *H. marginatum* ticks, 5 passages through mouse brains, 1 passage in PEK cells. Differs from the sequence (Romanova et al., 2007) by 2 synonymous mutations in positions 19 and 10104, and 2 nonsynonymous mutations in positions 2249 (E protein $Ile_{426} \rightarrow$ Ile/Thr) and 3300 (protein NS1 $Glu_{280} \rightarrow$ Asp), which did not affect variant properties.

Viruses were used as culture supernate from infected PEK cells.