SHORT COMMUNICATION

Anti-tick-borne encephalitis (TBE) virus neutralizing antibodies dynamics in natural infections versus vaccination

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One sentence summary: This work provides an increase on the knowledge of the laboratory investigation of TBE virus neutralizing antibodies through the analysis of a consistent number of individuals with a past Tick-borne encephalitis and vaccinated subjects.

ABSTRACT

Tick-borne encephalitis (TBE) virus infection elicits a life-long lasting protection. However, little is known about the neutralizing antibodies titres following natural infection. In this study, subjects with past TBE disease (\(n = 62\)) were analysed for the presence and titre of anti-TBE neutralizing antibodies, and compared with a vaccinated cohort (\(n = 101\)). Neutralizing antibody titres were higher in individuals with past TBE and did not show an age-dependent decrease when compared with vaccinees.

Key words: TBEV; immunity; plaque reduction neutralization test; vaccine; natural infection

THE STUDY

Tick-borne encephalitis (TBE) is an emerging viral disease of the central nervous system in Europe (Lindquist and Vapalahti 2008). This infection, which may result in long-term neurological sequelae in 26–50% of the patients, can be effectively prevented by active immunization (Gunther and Haglund 2005). In Europe, two TBE vaccines are available: FSME-Immun\(^\Reg\) (Baxter Innovations GmbH, Vienna, Austria) and Encepur\(^\Reg\) (Novartis Vaccines and Diagnostics GmbH & Co., KG, Marburg, Germany) (Gunther and Haglund 2005; Lehrer and Holbrook 2011). The protective immunity rate of the vaccines has been estimated to be 96–98% according to field studies in Austria (Heinz et al. 2007).

Serological follow-up studies in adults have revealed long-term persistence of protective immunity following at least one booster immunization (Rendi-Wagner et al. 2006). However, studies which analysed different age groups showed...
differences between younger (aged < 50 years) and older adults (aged ≥ 50 years) with respect to TBE antibody persistence (Hainz et al. 2005; Jilkova et al. 2009; Loew-Baselli et al. 2009; Paulite-Korinek et al. 2009, 2013; Baldovin et al. 2012). Moreover, it has been reported that vaccination breakthroughs are more frequent in the elderly (Stiasny, Holzmann and Heinz 2009). Accordingly, booster doses at 5- and 3-year intervals are currently recommended for those younger and older than 60 years, respectively (Rendi-Wagner et al. 2006; Heinz et al. 2007). Considering a population with 3-year booster interval for the over 60, it has been reported that the field effectiveness of TBE vaccination exceeded 97%, with no significant difference between the age groups (Heinz et al. 2007).

Anti-TBE immunity elicited by TBE natural infection is known to confer life-long protection against re-infection (Holzmann 2003). However, little is known about antibodies dynamics following natural infection. Recently, Baldovin et al. (2012) have shown that, while antibody levels detected by ELISA decreased significantly with increasing age in a vaccinated cohort, this was not observed in individuals who had developed TBE in the past. Low titres of anti-TBE neutralizing antibodies (NAbs) have been detected in patients with acute TBE, who were positive for IgM antibodies and had high haemagglutination-inhibition antibody titre. Higher anti-TBE NAb titres were instead detected in healthy subjects residing in a TBE endemic area, seropositive for TBE virus (TBEV; Venturi et al. 2009).

In the present study, most of the sera analysed belongs to the populations previously described by Baldovin et al. (2012), residing in the Belluno area (Veneto region, Northeast Italy), a mountainous region where TBE is highly endemic. The characteristics of populations are described in Table 1. Sera from 62 patients (age, mean ± standard deviation (SD): 54.67 ± 18.38; range 2.44–80.33), hospitalized between 1994 and 2007 with a diagnosis of TBE (number of years after disease, mean ± SD: 6.01 ± 3.60; range 1.47–13.76), and from 101 vaccinated subjects (age, mean ± SD: 51.59 ± 12.07; range 10.26–88.88), who had completed primary TBE immunization (years from the last vaccine dose, mean ± SD: 5.33 ± 1.13; range 0.66–8.82), were tested for the presence of NAbs by a plaque reduction neutralization test (PRNT). Informed consent was obtained from all participants before their enrolment. Vaccinates had received three doses of the vaccine FSME-IMMUN®/Tico-Vac® (Baxter Hyland Immuno, Vienna, Austria), containing 2.4 µg of TBEV/dose (Neudörfli strain). PRNT was performed as previously described using TBEV_JSS ir 454 strain (Venturi et al. 2006), with the exception that PK15 (BS-CL-72) cells were used. Spearman’s rank correlation coefficients (Spearman’s rho) were used to evaluate the correlation between PRNT titres and age, PRNT titres and age at the time of the last dose of vaccine or onset of TBE and PRNT titres and time elapsed since the last vaccine dose or TBE onset. Spearman’s rho is a non-parametric measure which assesses how well the correlation between two variables can be described using a monotonic function. The Kolmogorov–Smirnov non-parametric test was used to compare PRNT titres between vaccinees and naturally infected individuals; age, age at the time of the last dose of vaccine and the time elapsed since the last dose of vaccine between PRNT positive and negative subjects; PRNT titres between younger (<50 years old) and elderly (≥50 years old) participants, both vaccinated and naturally infected. The analysis was performed by using the statistical software Intercooled Stata 11. In addition, the presence of IgM antibodies was evaluated in 38 of 62 sera from naturally infected subjects (years from acute TBE disease: 4.48±3.58 mean ± SD, range: 1.47–13.76 years) by an ELISA test (FSME IgM Immunozym, Progen Biotech GMBH, Heidelberg, Germany), in order to verify their persistence in subsequent years after the disease (Holzmann 2003; Stiasny et al. 2012).

Among naturally infected subjects, the log10PRNT80 titres ranged from 5.32 to 12.32 (mean ± SD: 9.34 ± 1.73). Within the vaccinated group, 24 of 101 subjects had no detectable NAbs, while the remainders showed log10PRNT80 titres ranging from 3.32 to 11.32 (mean ± SD: 4.26 ± 2.78). The difference in NAb titres between the two populations was statistically significant (P = 0.0001).

Among vaccinates, there was no significant correlation between PRNT titres and the time from the last vaccine dose (Spearman’s rho = 0.096), while a significant negative correlation was observed between both PRNT titres and age (Spearman’s rho = −0.319), and between PRNT titres and age at the time of the last vaccine dose (Spearman’s rho = −0.326). As reported in Table 2, participants who had lost detectable NAbs (n = 24) were more likely to be older (P-value = 0.007), and to be older at the time of the last vaccine dose (P-value = 0.003), when compared with individuals with a positive PRNT titre (n = 77). However, the two groups did not significantly differ in the time elapsed since the last vaccination (P-value = 0.239).

Among naturally infected subjects, there was no significant correlation between PRNT titres and the time elapsed
Table 3. Comparison of the PRNT titres between young (<50 years old) and elderly (≥50 years old) vaccinated and naturally infected subjects.

<table>
<thead>
<tr>
<th>Age classes</th>
<th>PRNT titres (mean ± SD) Log2 NT80</th>
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<tbody>
<tr>
<td></td>
<td>Vaccines</td>
</tr>
<tr>
<td>Subjects</td>
<td>3.61 ± 1.39</td>
</tr>
<tr>
<td>&lt; 50 years</td>
<td>n = 43</td>
</tr>
<tr>
<td>Subjects</td>
<td>2.44 ± 2.12</td>
</tr>
<tr>
<td>≥50 years</td>
<td>n = 58</td>
</tr>
<tr>
<td>Kolmogorov-Smirnov Test</td>
<td>P-value = 0.022*</td>
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*significant test (the null hypothesis is rejected).

from TBE disease (Spearman’s rho = 0.07), and, differently from what observed in the vaccinees, between PRNT titres and age (Spearman’s rho = 0.162) or age at the time of clinical TBE (Spearman’s rho = 0.153).

We also compared the mean PRNT titres between younger (<50 years old) and elderly (≥50 years old) subjects, and found that, in the vaccinated group, younger subjects showed PRNT titres significantly higher than older individuals (P-value = 0.022) (Table 3). Interestingly, this was not true in the group of naturally infected subjects, where older individuals showed PRNT titres higher than the younger ones, even if the difference was not statistically significant (P-value = 0.638). The high titres detected in some naturally infected older individuals may be explained by the probability of a second contact with the virus, a possible event for this population that already had come in contact with the TBEV in the past.

Although it has been reported that, after TBE infection, TBE-specific IgM antibodies may be detectable for several months (Holzmann 2003; Stiasny et al. 2012), none of the 38 samples analysed by ELISA was found positive for the presence of anti-TBE IgM antibodies suggesting a past infection rather than a recent re-exposure.

To our knowledge, this is the first study which presents data on anti-TBE NABs from a consistent number of past TBE naturally infected subjects. Accordingly and in addition to our previous studies, where we analysed the NABs of TBE acute patients and of subjects with previous asymptomatic TBE infection (Venturi et al. 2009), the present work clearly identifies different NAB dynamics between natural infection and vaccination. The development of NABs in the acute phase of the disease is delayed compared with the rapid appearance following vaccination (Venturi et al. 2009). However, anti-TBE NAB titres are much higher among individuals who developed the disease than among those who were vaccinated, and they do not demonstrate an age-dependent decrease following natural infection. As anti-TBE immunity confers life-long protection following natural infection, our data further strengthen the NAB predictive value of protection. NAB dynamics in vaccinated subjects are consistent with those reported in the international literature: vaccinated subjects older than 50 show PRNT titres significantly lower than younger subjects. It must be noted that elderly subjects had completed the primary vaccination schedule from longer than younger subjects. It must be taken into account that, being TBEV vaccine a formalin-inactivated vaccine, a robust or prolonged neutralizing Ab response is unlikely with respect to a natural infection. This limit may possibly be overcome by different vaccination strategies or new vaccines built with innovative technology, such as the new single-cycle TBE vaccines which are currently under investigation (Rumyantsev et al. 2013; Koraka, Martina and Osterhaus 2010).

Conflict of interest statement. None declared.

REFERENCES


