Factors associated with seroimmunity against tick borne encephalitis virus 10 years after booster vaccination

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A B S T R A C T
In a sample of originally 430 healthy adults (18–84 years of age) with documented basic and booster immunization against tick borne encephalitis, cumulative seroprotection rates 8 (n = 178) and 10 years (n = 183) after the last booster dose were 86.8% and 77.3% according to the neutralization test, respectively. In subjects aged 50 years and older, antibody titers were significantly lower compared to subjects younger than 50 years. History of any allergy but not previous exposure to other flaviviral antigens was associated with higher neutralization titers. In subjects with waning immunity, a single booster dose induced a strong anamnestic antibody response.

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1. Introduction
Tick borne encephalitis (TBE) is the most frequent cause for viral meningitis in Europe [1–4]. In many areas it is an “emerging disease” [3,5]. There is no specific therapy to treat the viral infection or clinical disease. However, it can be effectively prevented by safe and effective vaccines, one of which has been available for more than 30 years [4,6,7]. Despite known high prevalence rates of the tick borne encephalitis virus (TBEV) in many regions in Europe, vaccination coverage rates are very low in most endemic countries except Austria [8]. Basic immunization against TBE usually consists of three vaccinations within 1 year, followed by another vaccination 3 years later and afterwards booster immunizations every 5 years according to national recommendations [1]. From the age of 60 years onwards, in Austria booster immunizations are scheduled every 3 years [1] due to the diminished capacity of the immune system in the elderly [9]. Intervals for boosters are discussed controversially, mainly due to the lack of long term seroimmunity data. Simplified vaccination schedules with extended booster intervals could improve compliance within vaccination programs in TBE-endemic regions.

Antibodies measured by neutralization tests (NTs) are surrogates for clinical protection against TBE [6]. Antibody levels after TBE vaccination were detectable up to 21 years in retrospective studies [10,11], and prospective studies demonstrated protective antibody levels up to 6 years after TBE vaccination in most vaccines [12,13]. In this study, for the first time prospective data on TBE-specific antibody levels up to 10 years after the last booster dose are analyzed.

Furthermore, antibody response in subjects with waning immunity 10 years after a TBE booster dose was tested after a single TBE vaccination. Moreover, possible determinants of the antibody levels obtained after 8–10 years such as age, history of allergies or preceding vaccination against Japanese Encephalitis or Yellow Fever were investigated. In addition, performance of a commercially available TBE-IgG-ELISA test, Enzygnost®HM, was compared with results of the NT.

2. Materials and methods
2.1. Study design and population
A sample of 430 healthy males and females with documented basic immunization against TBE (schedule 0–1–(9–12), FSME-Immun®, Baxter) and the last vaccination at least 3 years before study entry was recruited in 2002. Subjects were stratified for age at the time of the booster vaccination in groups of persons below
50 years of age, and subjects 50 years of age and older. Briefly, the subjects underwent antibody testing, were then boosted once with Encepur® adults (irrespective of the antibody titer before vaccination) and antibody titers were tested again 3 weeks after the booster dose. Afterwards, starting 2 years after the Encepur® booster, the study participants were invited annually for antibody testing. Results for visits up to 6 years post-booster have been published previously [4,10,12,14,15]. Following year 6, subjects were invited for antibody testing 8 and 10 years after the booster dose.

To compare the quantity and quality of the anamnestic response in subjects with only short lasting protective NT-titers versus those with obviously longer persistence of antibodies, subjects with NT titers of 10 or less 2 and 10 years after the booster, respectively, received another dose of FSME-Immunit® and the immune status was assessed again at an interval of 3–10 weeks after that additional booster dose in this subgroup.

2.2. Study population

Subjects were excluded from long term follow up if they had received an additional booster vaccination against TBE outside the study protocol. Furthermore, subjects were excluded if they suffered from any medical condition likely to impair antibody levels during the follow-up period.

Subjects with low antibody levels at years 2 and 10 post-booster were shifted to a subgroup, received a TBE booster dose and were then retested separately.

Information on preceding (prior to and during the follow-up study) vaccinations against Japanese Encephalitis or Yellow Fever was collected and subjects were evaluated with respect to history of flavivirus infection. History of allergies was collected in year 10 post-booster.

Study participants signed informed consent forms for every single follow-up blood draw. The study was conducted in compliance with the principles of the Declaration of Helsinki and the good clinical practice guidelines according to the International Conference of Harmonization, and ethics approval was obtained.

2.3. TBE antibody determination and SPR

Neutralization tests (NTs) were performed in all subjects; Enzygnost™ was conducted in all subjects without history of exposure to any other flaviviral antigen than TBE.

Between year 2 and year 6, NT was performed at Novartis Vaccines, Marburg, and has been described elsewhere [10,12,14,15]. For the current study results of these tests are used only for determination of seroprotection rates (SPR) (Fig. 1).

The NT applied in year 8 and year 10 was an in-house neutralization test at the Department of Virology, Medical University of Vienna and Austrian national reference laboratory for TBE [16,17]. Subjects with NT titers of >10 were considered protected. Test results with NT titers equal to 10 were defined as borderline [6,18,19].

In the subgroup of subjects with low antibody titers in year 2 (early booster, 14 subjects) and year 10, post-booster (late booster, 20 subjects) serum was obtained and stored at −20 °C for further analysis. To enable comparison of test results, sera after booster vaccination from 2 years post-booster and 10 years post-booster were tested with the same NT test system (Department of Virology, Medical University of Vienna).

Antibody levels were determined with Enzygnost™, a commercial ELISA test kit (Dade Behring, Marburg, Germany). Antibody levels of ≥10 U/mL were interpreted as positive test results.

2.4. Statistical analysis

Demographic data (mean age at time of blood draw, gender) were evaluated 8 and 10 years post-booster with descriptive statistics. Furthermore, SPRs and geometric mean titers (GMT) were calculated.

For comparison of the log-NT-titers in the different age groups analysis of variance and of subjects with/without history of Japanese Encephalitis or Yellow Fever vaccination and subjects with/without allergy, the Student’s t-test was applied. In addition, multivariate linear regression with age, gender, number of previous vaccinations, history of allergies and contact with other flaviviruses except TBE was performed. Statistical significance was assumed if p-values were <0.05. Analysis of data was performed with Graph Pad Prism 4 and Stata 12.

3. Results

3.1. Demographics

Eight and 10 years after TBE booster vaccination, 178 and 183 subjects returned for blood draws, respectively, giving participation rates of 41% and 43%. Demographics are shown in Table 1.

Table 1: Demographic data characterizing the study population that returned for a blood draw 8 and 10 years after the last booster immunization in 2002.

<table>
<thead>
<tr>
<th></th>
<th>Year 8</th>
<th>Year 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>178</td>
<td>183</td>
</tr>
<tr>
<td>Female (%)</td>
<td>66.3</td>
<td>66.1</td>
</tr>
<tr>
<td>Subjects &lt;50 years of age (%)</td>
<td>59.6</td>
<td>65.6</td>
</tr>
<tr>
<td>Subjects 50–60 years of age (%)</td>
<td>21.9</td>
<td>18.6</td>
</tr>
<tr>
<td>Subjects ≥60 years of age (%)</td>
<td>18.5</td>
<td>15.9</td>
</tr>
<tr>
<td>Mean age at time of vaccination (min–max)</td>
<td>43.9 (18.3–75.9)</td>
<td>42.1 (18.3–74.6)</td>
</tr>
<tr>
<td>Mean number of prior vaccinations (min–max)</td>
<td>5.8 (3–11)</td>
<td>5.8 (3–11)</td>
</tr>
<tr>
<td>Mean interval between last vaccination and 2002 booster (min–max)</td>
<td>6.2 (3–19.9)</td>
<td>6.3 (3–19.9)</td>
</tr>
<tr>
<td>Vaccination against Japanese Encephalitis</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>Vaccination against Yellow Fever</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Vaccination against Yellow Fever and Japanese Encephalitis</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2
Neutralization test geometric mean titer (NT-GMT) in subjects aged <50, 50–60 and 60 years of age and older.

<table>
<thead>
<tr>
<th></th>
<th>&lt;50 years</th>
<th>50 to &lt;60 years</th>
<th>≥60 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n year 8</td>
<td>106</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td>NT-GMT 8</td>
<td>72.1 (60.2–86.2)</td>
<td>25.5 (19.2–33.9)</td>
<td>38.7 (29.8–50.4)</td>
</tr>
<tr>
<td>n year 10</td>
<td>120</td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>NT-GMT 10</td>
<td>51.6 (42.6–62.6)</td>
<td>22.0 (15.1–32.2)</td>
<td>24.7 (17.6–34.6)</td>
</tr>
</tbody>
</table>

3.2. Seroprotection rates and geometric mean titers

Eight years after the booster vaccination, one subject had an NT titer <10, 11 subjects had NT titers of 10 and 166 subjects had titers >10 in the NT. Ten years after the booster, 14 subjects had NT titers <10, 11 subjects had NT titers of 10 and 158 subjects had titers >10 in the NT. SPRs were 93.3% (year 8) and 86.3% (year 10), respectively, according to the NT test in the investigated subjects.

Extrapolated for the total study population of 430, 57 subjects were tested negative in the NT during the 6 years period between years 2 and 8 post-booster, while between years 8 and 10 another 41 subjects showed titers ≤10.

The cumulative SPRs (subjects with titers >10) for the total study population were 86.8% and 77.3% at years 8 and 10 after the booster, respectively. In subjects aged 50 to <60 and subjects ≥60 years of age, 10 years post-booster, SPRs were 66.0% and 66.3%, respectively (Fig. 1).

The NT-GMTs for subjects <50, 50–60 and ≥60 years of age are summarized in Table 2.

NT antibody levels were significantly higher in the group of subjects <50 years of age compared to subjects 50 to <60 years of age at 8 years (p < 0.0001) and 10 years (p < 0.0001) after the booster, and this also holds for the age group of subjects <50 years compared to those ≥60 years of age (8 years, p = 0.0006; 10 years, p = 0.0007). No significant difference was obtained for the comparison of the age groups 50 to <60 years and ≥60 years.

3.3. Comparison of NT results with Enzygnost™ results

In 138 and 140 subjects Enzygnost™ results were evaluated for years 8 and 10 post-booster. According to the Enzygnost™ test in the 138 subjects 8 years after the booster dose, the GMT was 130.4 U/mL (95% CI 112.4–151.3). In 140 subjects 10 years post-booster, the GMT according to Enzygnost™ was 100.2 U/mL (95% CI 83.8–119.8).

In total, test results from 278 samples were available for both, NT and Enzygnost. Comparing the results of the NT test with Enzygnost™, test results showed that even though 30 test results were NT ≤10, only one sample showed an Enzygnost™ titer <10 U/mL. All samples with protective titers in NT were also positive with Enzygnost™.

3.4. Subjects with waning antibody titers 2 and 10 years after booster vaccination

In year 2, 14 subjects of the 195 tested were re-vaccinated because of low antibody titers (early booster). Their mean age was 59.7 years (SD 8.36). Ten years after the booster, in 25 subjects (of the 183 tested) NT antibody levels decreased to 10 or less. Among them, 20 volunteers received a TBE booster dose (later booster; 5 subjects did not return for a booster dose and blood draw). The mean age of these subjects was 59.8 years (SD 11.24).

The two different groups were analyzed to obtain information on boosting properties: post-booster NT-GMTs in the 14 subjects vaccinated in year 2 (early booster) was 58.0 (95% CI: 40.0–84.1). In the subjects who were vaccinated 10 years post-booster (later booster), NT titers were between 30 and 1280; the NT-GMT was 192.0 (95% CI: 123.6–298.3). The difference in log-NTs between these groups (early and later booster) was highly significant (p = 0.0002).

3.5. History of exposure to flaviviral antigens

Eight and ten years after the booster dose, history of Japanese Encephalitis vaccination was reported in 24 subjects of the study population (year 8: 21 subjects; year 10: 23 subjects), and 16 subjects had been vaccinated against Yellow Fever (year 8: 13 subjects; year 10: 16 subjects). Another 6 persons had received both vaccinations (year 8: 6 subjects; year 10: 4 subjects). None of the participants reported any history of infection with a virus of the family Flaviviridae. In the 40 (year 8) and 43 (year 10) subjects with history of flavivirus-vaccination other than TBE, the NT-GMT was 45.1 (95% CI: 34.6–58.9; n = 40) and 34.9 (95% CI: 25.9–46.9; n = 43). In the group of subjects without any preceding flavivirus vaccination, NT-GMT was 53.3 (95% CI: 44.7–62.9; n = 138) and 40.7 (95% CI: 33.62–49.1; n = 140) at years 8 and 10 post-booster, respectively. There was no significant difference in log-NTs in the two groups neither (p = 0.362) nor 10 years (p = 0.426) after the booster vaccination.

3.6. Influence of allergies on antibody titers

Ten years post-booster, history of allergies was evaluated. There were 61 subjects who reported chronic or seasonally recurrent allergic disease including allergies against pulmonary allergens, food allergens, drug allergies and contact allergens. In this group the NT-GMT was 51.3 (95% CI: 38.1–69.1) compared to 34.3 (95% CI: 28.4–41.3; n = 122) in subjects who did not report any kind of allergy. Age was not significantly different in the 2 groups (allergy-group mean age 39.6 (SD 13.13); no-allergy-group 43.36 (SD 14.76); p = 0.0979), but the difference in log-NTs in the 2 groups was statistically significant (p = 0.0197). In the multivariate regression analysis of log-NT on age, sex, previous vaccination against other flaviviruses and allergies only age and allergies had a significant influence with age reducing the titer (p = 0.002) and allergies increasing the titer (p = 0.035).

4. Discussion

This prospective long term follow up study after TBE vaccination documents the first time the antibody persistence 8 and 10 years after TBE booster vaccination.

Throughout 8 years post-booster, the extrapolated cumulative SPR in our study population still was 87%, indicating that post-booster TBE antibodies tend to persist at least for this period to a sufficient manner. However, this pattern significantly changed at year 10 after the booster dose: in the last 2 years of the follow-up in another 9.5% of the subjects, antibody levels declined below the threshold of protection implying the immediate need for a booster dose against TBE. This is nearly the same number of subjects losing their antibodies within a 2 years period compared to the 8 years period before. According to the already published results of post-booster SPRs over time, where after 3, 4, 5 and 6 years only sporadic and very rarely subjects fell under the protective
threshold [12,14,15], this increase of persons needing boosters in
the period 8–10 years post-booster is striking and it may well be
speculated that this can be interpreted as an upcoming end of the
regular protection period after a TBE booster. However, it must be
considered that subjects with low antibody titers after their first
booster in the study were more likely to return for blood draws
because they were more worried of losing their protection against
TBE and therefore this population is over-represented [12]. There
is no unique accepted definition of seroprotection. However, ac-
cording to WHO [6] NT tests are considered to be adequate surrogates
for protection. In this study, we defined the threshold of protection
at NT levels >1:10. Several studies on antibody levels after primary
TBE vaccination [20,21] and follow-up until 6 years post-booster
showed that TBE antibody levels were generally lower in the elderly
[12,13,15,22–24] and this is also shown in the present investigation
until year 10. More precisely, signs of immunosenescence repre-
sented by significantly lower antibody levels, were detected not
only in persons aged 60 years and older, but also in those aged
50–60 years old. This indicates that boosting recommendations
have to be adapted to populations younger than 50 and persons
aged 50 years and older. However, for TBE-vaccinations and also
for other vaccinations, it has been demonstrated that immuno-
senescence becomes critical for vaccination response after 60 years
of age [21,25].

4.1. Comparison of NT results with Enzygnost™ results

Enzygnost™ test was only performed in subjects without pre-
vious exposure to any flaviviral antigen to rule out false-positive
results by cross-reactive antibodies [17,26]. However, NT showed
borderline or negative results (NT ≤ 10) in 30 of these subjects,
but Enzygnost™ test results implied non-protective antibody lev-
eels in only one subject. From our results it can be concluded that
NT, which is the most specific test to detect TBEV-specific anti-
bodies [26], is highly specific at the expense of limited sensitivity.
Therefore, NT test results can be assumed to be safe and very con-
servative. Vice-versa, Enzygnost™ is highly sensitive, but with
this method also non-neutralizing antibodies against TBE can be
detected giving this ELISA high sensitivity but limited specificity.
However, since neutralizing antibodies are a prerequisite for pro-
tection against TBE [6], NT should be preferred. Furthermore, this
commercial test system seems to have only very limited predic-
tive power for information on the duration of protection after TBE
vaccination.

4.2. Subjects with waning antibody titers 2 and 10 years after
booster vaccination

Subjects whose antibody titers fell below the protective thresh-
old 2 and 10 years post-booster, were boosted and to ensure re-
established protection, antibody levels were evaluated again
after the booster dose in the same test system. Comparison of post-booster-antibody titers in persons who were vaccinated
because of low antibody titers 2 versus those 10 years after
the last booster dose, respectively, showed significantly higher
post-booster log-NTs in those who were vaccinated 10 years
post-booster. This indicates two findings. First, an anamnestic
response can be triggered even after a very long period (in
our case 10 years). Even though antibody levels may decrease
below the protective threshold in these subjects after a pro-
longed period, re-vaccination is followed by a very pronounced
anamnestic immune response leading to high post-booster anti-
body titers. In contrast, those who needed the booster dose very
early because of low antibody titers (2 years after the last vac-
cination) produced significantly lower antibodies. Therefore, it
is tempting to speculate, as suggested from other vaccines like
hepatitis A and B [27,28], that even for TBE there may be a
small proportion of “low responders”, where post-booster anti-
body titers tend to be lower and additional boosters cannot
overcome this attribute. This feature seems to be independent of
age.

4.3. History of exposure to flaviviral antigen

It is suggested that different flaviviral antigens are cross-reactive
and would, at least to some extent, cause cross-protection [29–31].
Considering this issue, the influence of preceding exposure to TBE
antigen on post-vaccination antibody titers was assessed regarding
antibodies against Japanese Encephalitis in persons vaccinated
against TBE [29]. However, in this study after two vaccinations
against Japanese Encephalitis, there was no significant differ-
ce in antibody titers against Japanese Encephalitis; antibodies
against TBE were not measured [29]. Though, this was addressed
in the current investigation. Yet, TBE antibody levels in the NT
test were not influenced by exposure to Japanese Encephali-
is or Yellow Fever vaccine upon comparison of subjects with
and without history of contact to flaviviral antigen other than
TBE.

4.4. Influence of allergies on antibody titers

In the current investigation, adults who reported any kind of
allergy including atopy and anaphylactic allergies showed signifi-
cantly higher antibody levels compared to persons who did not
report any kind of allergy. This is in contrast to studies where anti-
body levels in association with allergy were assessed after tetanus
and pertussis vaccination in atopic children compared to healthy
children. In those studies, there was no significant difference in the
levels of antibodies after vaccination [32,33]. The higher antibody
levels in allergic patients which were detected in our investiga-
tion could be explained by a generally abnormal, increased and
inappropriate immune response in allergic patients [34], but a
detailed explanation cannot be provided. The high activity of the
immune system in allergy patients could resemble an unspecific
stimulus to the immune system like it is discussed for infectious
diseases, maintaining high antibody levels [35], also against TBE.
Another explanation could be high rates of IgG4 antibodies in aller-
gic patients compared to non-allergic patients, increasing the total
levels of antibodies detected. However, the rate of IgG4 antibodies
has not been assessed in the current investigation and should be
further addressed.

5. Conclusion

Summarizing our findings, seroprotection will last up to 8 years
in most of the persons with 4 or more preceding TBE vaccinations.
However, between years 8 and 10 post-booster, a disproportio-
nately high percentage of subjects lost protective antibody titers.
Taking into account that our study population consisted of healthy
and supposedly fully immuno-competent individuals, our results
have to be carefully evaluated with respect to implications for gen-
eral extended booster recommendations: like with other vaccines,
an ample safety buffer in future booster recommendations will
definitely be necessary. Immunosenescence and – a very small –
proportion of “low responders” are also a challenge for standard-
ized recommendations for booster intervals.

Again, TBE vaccination demonstrated to be highly immunogenic
and the boosting properties in those who lost their antibodies dur-
ing the observation period clearly indicate that this kind of vaccine
establishes a strong immune memory.
Acknowledgement

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Conflict of interest: Maria Paulke-Korinek accepted fees for speaking and received funding to attend conferences from Novartis. Martin-ponders has received advisory board and consultancy fees from Novartis and Baxter. Herwig Kollaritsch accepted educational grant fees and payment for lectures, for serving on advisory boards and as an independent safety monitor in clinical studies, and reimbursement for attending meetings from Novartis. The authors have no other relevant affiliations or financial involvement with the subject matter or materials discussed in the manuscript.

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