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Quantitative determination of IgM antibodies reduces the pitfalls in the serodiagnosis of tick-borne encephalitis

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ABSTRACT

Background: Tick-borne encephalitis (TBE) is the most important arbovirus disease in parts of Europe and Asia. Its laboratory diagnosis depends on the detection of specific IgM antibodies which can be impeded by (1) long-time persistence of IgM antibodies after infection, (2) vaccine-induced IgM antibodies, and (3) cross-reactive IgM antibodies from other flavivirus infections.
Objectives: To assess the extent of interference factors in the serodiagnosis of TBE that might lead to the false positive assignment of a recent infection.
Study design: We quantified TBE virus-specific IgM and IgG antibodies in sera collected at different time points from cohorts of (1) 61 TBE patients, (2) 131 TBE vaccinees, and (3) 42 patients with recent dengue or West Nile virus infections.
Results: All of the TBE patients were IgM- and IgG-positive upon hospitalization and 87% of acute TBE sera had IgM antibody titers of >500 Arbitrary Units (AU). These titers rapidly declined and only 16% of TBE patients had low IgM titers ≥9 months after infection. Vaccine-induced as well as flavivirus cross-reactive IgM antibodies were rarely detectable and of low titer.
Conclusions: Most of the potential problems of TBE serodiagnosis can be resolved by the quantification of IgM antibodies in a single serum sample taken upon hospitalization. High IgM values (>500 AU in our assay) are indicative of a recent infection. Lower IgM values, however, may require the analysis of a follow-up sample and/or a specific neutralization assay to exclude the possibilities of IgM persistence, vaccine-induced IgM antibodies or heterologous flavivirus infections.

1. Background

Tick-borne encephalitis virus (TBEV), like the closely related yellow fever (YF), Japanese encephalitis (JE), West Nile (WN) and dengue (DEN) viruses, is a member of the genus Flavivirus in the family Flaviviridae.[1] It is endemic in large parts of Europe as well as Central and Eastern Asia and >10,000 clinical cases of TBE are reported annually.[2]

In Europe, TBE usually takes a biphasic clinical course and starts with a primary phase of an uncharacteristic febrile illness ~7–14 days after infection.[3,4] After an asymptomatic interval of several days (range 1–21 days),[5] a second phase with central nervous system symptoms occurs in 20–30% of those infected, and this is generally the time point when the patients are hospitalized.[3,5,6] At this stage the virus is no more detectable by PCR in serum or cerebrospinal fluid in most of the patients,[7] but TBEV-specific IgM and IgG antibodies are already present at the beginning of the second phase.[6] The detection of specific IgM antibodies is therefore the standard parameter for the definitive diagnosis of a recent TBEV infection.[5,6,8]

Highly effective inactivated vaccines are available for the immunoprophylaxis of TBE, with strong variations of vaccination rates in different TBE-endemic countries.[9] The immunization schedule consists of a primary vaccination (two doses within a month), a third vaccination after 6–12 months and further booster vaccinations recommended at varying intervals in different countries.[2]

Three major problems impede the standard IgM-based serodiagnosis and may lead to falsely positive assignments of recent TBEV infections: (1) IgM antibody persistence after TBEV infection,[10] (2) IgM antibody persistence after TBE vaccination [11] and (3)

Abbreviations: TBEV, tick-borne encephalitis virus; DENV, dengue virus; WNV, West Nile virus; JEV, Japanese encephalitis virus; YFV, yellow fever virus.

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cross-reactivity of IgM antibodies caused by other recent flavivirus infections, either induced by a co-circulating flavivirus like WN virus (WNV) in certain parts of Europe and Asia [5,12,13] or in patients returning from areas endemic for other flaviviruses such as DEN virus (DENV).[6] To which extent these factors contribute to the problems of TBE serodiagnosishas not yet been addressed by a quantitative analysis of antibody titers.

2. Objectives

It was the goal of our study to assess the extent of IgM antibody persistence after TBEV infection and TBE vaccination as well as the degree of cross-reactions of antibodies induced by other flavivirus infectionsas problems for IgM-based TBE serodiagnosis. For this purpose, we quantified TBE IgM and IgG antibodies at different time points after acute infections and vaccination and determined the amount of TBEV cross-reactive antibodies in sera from recent DENV and WNV infections.

3. Materials and methods

3.1. Human sera

TBEV post-infection sera were obtained from 24 TBE patients admitted to the Hospital Ceske Budejovice at hospitalization as well as 3 and 9 months later (cohort 1). Sera from a second cohort of 37 TBE patients were collected ≥1.5 years (median 21 months; range 18–46 months) after admission to the same hospital (cohort 2).

TBE post-vaccination sera were collected after the two doses of the primary vaccination and after the 1st booster vaccination (3rd dose) from 131 healthy adults enrolled in two TBE immunization studies conducted at the Department of Virology, Medical University of Vienna and the University Hospital of Zurich with an inactivated vaccine (FSME-IMMUN® 0.5 ml, Baxter).

30 DENV and 12 WNV acute post-infection sera from Brazil and USA, respectively, had originally been submitted for diagnostic analyses to the Department of Virology, Medical University of Vienna.

3.2. TBE ELISA

TBEV-specific IgM antibodies were analyzed by an IgM capture ELISA and TBEV-specific IgG antibodies by a 3-layer ELISA as described previously.[14,15] Sera were quantified in Arbitrary Units (AU) with a standard polyclonal human anti-TBEV serum set at 1000 AU. Two-fold serial dilution curves of the standard (7 data points) were fitted using a four-parameter logistic regression KCjunior™. The definition of cut-offs for IgM (100 AU)and IgG (220 AU) antibody titers was based on the re-validation of the assays with 45 and 90 flavivirus negative sera, respectively.

3.3. DEN and WN ELISA

The following diagnostic ELISA kits were used for the detection of WNV- and DENV-specific IgM and IgG antibodies according to the manufacturer's (InBios) instructions: (1) DENV and WNV Detect™ IgM Capture ELISA and (2) DENV and WNV Detect™ IgG ELISA.

3.4. Statistical analyses

Statistical analyses were performed with GraphPad Prism 5 (GraphPad Software Inc.). Logarithmic transformation of the data was carried out to obtain approximate normal distribution of antibody concentrations. Two-tailed *t*-tests were applied to the transformed data for significance testing and *P* values <0.05 were regarded as statistically significant.

4. Results

4.1. Persistence of IgM antibodies after TBEV infection

To perform a quantitative analysis of TBEV-specific IgM antibody decline after infection, we tested sera obtained upon hospitalization (1st samples) as well as 3 months and 9 months later from a cohort of 24 TBE patients. IgM antibodies were found in all 1st samples, although the quantities observed were highly variable (Fig. 1A and Table 1) and three of the acute sera were only in the lower positive range of 250–500 AU (Fig. 1A). IgM antibody concentrations were below the cut-off (100 AU) in most of the follow-up samples (Fig. 1A), but two samples were still IgM-positive even after 9 months (Fig. 1A and Table 1). In the first of these cases, IgM antibodies had increased from 899 AU at hospitalization to 1080 AU 3 months later (Fig. 1A) and still yielded 331 AU 9 months after disease onset. In the second case, IgM antibodies dropped from 765 AU in the first sample to 202 AU after nine months (Fig. 1A); a 3-month sample was not available from this patient.

All of the samples collected upon hospitalization were not only IgM- but also IgG-positive (Table 1). The mean IgG antibody concentrations were similar upon hospitalization and 3 months later and slightly, but significantly, reduced after 9 months (Table 1 and Fig. 1B).

We also analyzed samples collected ≥1.5 years after disease onset from a second cohort of TBE patients. IgM antibodies were still detectable at this late time point in 22% of the cases (Table 1). The difference relative to the 9-month samples of cohort 1 was statistically not significant (*t*-test; $P = 0.12$). The longest interval between disease onset and a still positive IgM result was 32 months. A combined analysis of the samples obtained ≥9 months after hospitalization of both cohorts revealed that IgM antibodies were still detectable in 16% of the cases (Table 1). The values, however, were low with a maximum of 331 AU (Fig. 1C).

4.2. Persistence of TBEV-specific IgM antibodies after vaccination

For the investigation of vaccine-induced IgM antibody responses, sera obtained after the two doses of primary immunization as well as after the third dose (1st booster) were tested in ELISA. With respect to IgG antibodies, a stratification of the vaccines according to their age was performed to allow comparison with previous studies (Table 2). In both age groups, IgM antibody values were very low. Only one IgM positive sample was identified after the first dose and three after the second dose of the primary vaccination in vaccinees aged >50 years (Fig. 2A and Table 2). One of these three positives was derived from the same vaccine already positive after the first dose (Fig. 2A). 6–8 months after primary immunization, not a single serum yielded a positive IgM result (Fig. 2A and Table 2).

The lower IgG seroconversion rates and the significantly lower antibody concentrations of elderly vaccinees (*t*-tests; *P* values <0.05) (Table 2) were in good agreement with published data (Table 2)[16–19] as were the significantly higher antibody concentrations in both age groups after the 3rd dose compared to those after primary vaccination (Fig. 2B).

4.3. Flavivirus cross-reactivity

In order to evaluate interference by heterologous flavivirus cross-reactivity, we examined DENV and WNV post-infection sera in the TBE IgM and IgG ELISAs. All of the sera were IgM-positive in the corresponding homologous IgM assays (Table 3). None of the