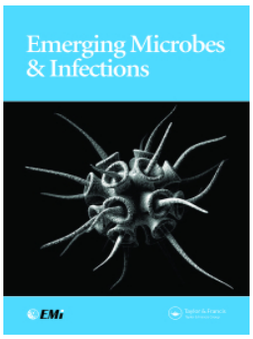

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Poor virus-specific T-cell responses early after tick-borne encephalitis virus infection correlate with disease severity

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ABSTRACT

Tick-borne encephalitis virus (TBEV) infection may cause acute central nervous system inflammation varying in clinical manifestations and severity. A possible correlation of TBEV-specific antibody and cell-mediated immune responses, shortly after infection, with clinical manifestations, severity and long-term outcome has been poorly investigated. In a cohort of thirty early tick-borne encephalitis (TBE) patients, we assessed the magnitude, specificity and functional properties of TBEV-specific T-cell and antibody responses. These responses early during disease were assessed in view of clinical manifestations, severity and long-term outcome. TBEV-specific T-cell responses to C, E, NS1, and NS5 proteins were significantly lower in patients with severe acute illness than in patients with mild TBE. Lower T-cell responses to E, NS1, and NS5 proteins also correlated with the development of meningoencephalomyelitis. Virus-specific antibody titres early after infection did not correlate with disease severity, clinical manifestations, or long-term outcome in this study, possibly due to the small number of patients of which matching serum and peripheral blood mononuclear cells were available. The findings suggest that virus-specific T cells afford a certain degree of protection against the development of severe TBEV-induced disease.

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KEYWORDS TBEV; Tick-Borne Encephalitis (TBE); TBEV-specific T-cells; disease severity; outcome

Key points:

- Poor TBEV-specific T-cell responses early during the course of acute illness correlated with TBE severity.
- Magnitude of virus-specific T-cell responses early after TBEV infection inversely correlated with the risk of developing meningoencephalomyelitis.

Introduction

Tick-borne encephalitis (TBE) is an important neurological disease that occurs in endemic areas from Europe to Far-Eastern Asia [1]. The disease is caused by tick-borne encephalitis virus (TBEV), a member of the *Flaviviridae* family and is mainly transmitted through the bite of infected ticks [2]. The spread of the disease in previously non-endemic and endemic regions of Europe and Asia is increasing with up to 15,000 cases reported annually [1–3]. Most human cases are caused by the European, Siberian and Far-Eastern subtypes of TBEV [3,4].

Outcomes of TBEV infection vary widely from asymptomatic infection to severe disease with fatal outcome or long-term neurological sequelae, which may depend on host factors, like age, gender, co-morbidities, immune status, and genetics on the one hand, and TBEV strain and dose on the other [5]. Viruses of the European TBEV subtype typically cause a biphasic course of disease, with an initial viraemic phase associated with non-specific symptoms such as fever, malaise, fatigue and headache, followed by a brief asymptomatic period and a second phase in which patients may remain asymptomatic or present with neurological disease. Neuronal TBE manifestations may include meningitis, meningoencephalitis or meningoencephalomyelitis [4,6–8]. Importantly, long-term post-encephalitic sequelae can occur in

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about a third of symptomatic TBE cases [9]. Antiviral treatment against TBEV is not available and besides non-pharmaceutical measures, vaccination is the protective measure of choice. However, for currently used vaccines repeated vaccinations are required. Furthermore, vaccination breakthrough infections are not uncommon [10,11].

Factors that underlie disease manifestations and severity of TBE are poorly understood. On the one hand, infection of the central nervous system by TBEV with direct neuronal damage may contribute to the disease. On the other hand, the virus-specific immune response induced upon TBEV infection, may either contribute to viral clearance, thus leading to protective immunity or instead, may contribute to immune-mediated pathogenesis. It has been suggested that cell-mediated immune responses contribute to protection against infection, because the peak of the CD4+ and CD8+ T-cell responses was shown to coincide with the clearance of the virus early after infection [12]. Also, the induction of virus-specific serum antibodies of the IgM and IgG isotypes, detectable from the end of the first phase of TBE, may contribute to viral clearance and protection [7].

Taken together, our knowledge on the relative contribution of cell-mediated and humoral immune responses to protective immunity or pathogenesis of TBEV infection is limited. Little is known about the specificity and functional properties of virus-specific T-cells, induced after infection and their effect on the short- and long-term outcome of the infection. The same holds true for the virus-specific antibody response. For example, it has been shown in a mouse model that in addition to virus-neutralizing antibodies, antibodies to non-structural protein 1 (NS1) contribute to protective immunity [13,14], but their role in human infections has not been conclusively demonstrated. We hypothesize that virus-specific cellular and possibly humoral responses induced early during TBEV infection determine clinical course of TBE, including its severity and long-term outcome. To this end, we determined the magnitude, specificity, and functional properties of the virus-specific T-cell and antibody responses in a cohort of TBE patients with various disease severity scores and clinical presentations during acute illness and at one year follow-up. Peripheral blood mononuclear cells (PBMC) isolated early in the course of TBE were used to determine and characterize the T-cell responses to overlapping peptide pools spanning structural and non-structural proteins. Serum samples were collected to characterize the virus-specific antibody response. The findings provide valuable information on the identification of correlates of development of severe disease, clinical presentation with paresis (meningoencephalomyelitis) and the subsequent development of long-term post-encephalitic symptoms, including neurological sequelae.

Materials and methods

Study subjects

Thirty patients diagnosed with TBE, hospitalized at the University Medical Centre Ljubljana, Slovenia were enrolled. Recent TBEV infection was confirmed by the presence of TBEV-specific IgM combined with IgG serum antibodies. The selected patients were categorized by the clinical manifestations of TBE, defined as meningitis, meningoencephalitis or meningoencephalomyelitis. Patients who manifested signs and symptoms of meningeal inflammation were categorized as having meningitis. Meningoencephalitis was diagnosed when, in addition to meningeal inflammation, patients displayed symptoms or signs of brain tissue damage including, but not limited to, tongue fasciculation, tremor of extremities, altered consciousness, diminished cognitive abilities and concentration, and/or seizures. When patients also displayed flaccid paresis, they were diagnosed having meningoencephalomyelitis (Table 1). Quantitative assessment of disease severity of acute TBE was performed using standardized questionnaire for 21/ 30 (70%) of the patients. The clinical score represented the sum of scores given to individual specific signs and symptoms of TBE. Clinical scores ranging from 0 to 8 were considered mild, whereas scores ranging from 9–22 and scores > 22 were considered moderate and severe respectively, as described previously [15] (Table 1).

The patients were followed-up for another year after TBE to monitor the long-term outcome of the

Table 1. Socio-demographic and clinical characteristics of 30 patients with tick-borne encephalitis.

Age (Median, range)	59.5, 28–88
Gender (M), %	15, 50%
Duration (sample collection – diagnosis) (Median, range)	4 days, 0–10
Previous vaccination for TBEV, JEV or YF	0
Immunosuppression (methotrexate) at time of sampling	2, 6.7%
Blood leukocyte count (x 10^9 cells /L; median, range)	9.5 (6.3–18.6)
Serum CRP level (mg/L; median, range)	8.5 (3–50)
CSF findings	
Leukocyte count (x10^6/L; median, range)	43.5 (6–512)
Protein level (g/L; median, range)	0.8 (0.34–2.12)
Clinical presentation	
Meningitis (M), %	4, 13.3%
Meningoencephalitis (ME), %	21, 70.0%
Meningoencephalomyelitis (MEM), %	5, 16.7%
Severity score (available for 21/ 30 patients)	
Mild* (0–8)	3, 14.3%
Moderate** (9–22)	12, 57.1%
Severe*** (>22)	6, 28.6%
Outcome (1 year after TBE diagnosis) (available for 29/ 30 patients)	
Favourable	16, 55.2%
Subjective symptoms affecting quality of life	8, 27.6%
Objective neurological sequelae	5, 17.2%

*All 3 patients had meningitis; **10 patients had meningoencephalitis, 2 meningoencephalomyelitis; ***4 patients had meningoencephalitis, 2 patients had meningoencephalomyelitis.

disease. The presence of subjective pre-defined TBE-associated symptoms and/or objective neurological sequelae one year after acute phase were considered as unfavorable outcome, as described previously [9]. Patients without these symptoms were categorized as having favorable clinical outcomes (Table 1). Details of collection of serum and PBMCs and statistical analyses used are described in supplementary material and methods.

Analysis of virus-specific antibody and T-cell responses

The presence of TBEV-specific serum antibodies was assessed by enzyme-linked immunosorbent assay (ELISA), Luciferase immunoprecipitation system (LIPS) and virus neutralization assay. Virus-specific T-cells were detected by IFN-γ ELISpot assay and flow cytometry. Details of these assays are described in the supplementary materials and methods.

Ethics statement

This study was approved by the Medical Ethics Committee of the Republic of Slovenia (No 152/06/12 and No 0120-467/2017/3). Written informed consent was obtained from each participant and the study was conducted in accordance with the 1975 declaration of Helsinki.

Results

Severity and clinical manifestations of TBE correlate with reduced frequencies of TBEV C-, E-, NS1- and NS5-specific T-cells

Of the TBE patients selected for in-depth immunological analyses, the majority had presented with meningoencephalitis (70%) followed by meningoencephalomyelitis (16.7%) and meningitis (13.3%) (Table 1). Apart from categorizing the clinical manifestations, also a quantitative severity score was used for most patients (Table 1). We first wished to investigate TBEV-specific T-cell responses early after diagnosis of TBE and determine the correlation of these responses with disease severity. To this end, we assessed the magnitude of TBEV-specific T-cell responses by IFN-γ ELISpot assay and stimulation of PBMCs with overlapping peptide pools spanning structural (C and E) and non-structural (NS1, NS3 and NS5) TBEV proteins. Interestingly, the frequency of C-, E-, NS1- and NS5-specific T-cells inversely correlated with disease severity score (Figure 1(A) and supplementary Figure 1). Likewise, the frequency of IFN-γ producing T-cells specific for the E, NS1 and NS5 proteins were significantly lower in patients with meningoencephalomyelitis than in patients with meningitis, which is considered a milder form of TBE (Figure 1(B)). No significant difference in the frequency of IFN-γ producing T-cells specific for E and NS5 was observed between patients with meningitis and meningoencephalitis (Figure 1(B)). These observations suggest that impaired TBEV-specific T-cell responses correlated with TBE severity and the development of meningoencephalomyelitis early after infection. Thus, poor T-cell responses may be the cause or the consequence of more severe clinical course of acute TBE illness.

Cytokine expression profiles of CD4+ and CD8+ T-cells specific for structural or non-structural TBEV proteins in TBE patients.

Polyfunctional T-cells are known to contribute to viral clearance and protection against severe disease [12,16]. Because the ELISpot assay does not allow discrimination between T-cell subsets, we further characterized responding TBEV-specific T-cells with multiparametric flow cytometry. To this end, PBMCs were stimulated with peptide pools spanning TBEV structural proteins C and E or non-structural proteins NS1, NS3 and NS5, and production of IFN-γ, IL-2 and TNF-α was analyzed after exclusion of naïve T-cells. Representative dot plots depicting intracellular cytokine production are shown in Figure 2(A). The frequency of IFN-γ+CD4+ T-cells specific for structural proteins C and E were significantly lower in patients with meningoencephalomyelitis, which was in concordance with results obtained in the ELISpot assay (Figure 2(B)). However, frequencies of TNF-α+CD4+ or IL-2 + CD4+ or frequencies of CD8+ T-cells producing any of the cytokines tested upon stimulation with peptides derived from the structural proteins were not different between groups (Figure 2(B)). In contrast, the frequency of IFN-γ+CD8+ T-cells directed to non-structural proteins NS1, NS3 and NS5 were significantly higher in patients with meningitis than in patients with meningoencephalomyelitis (Figure 2(C)). This difference was not observed for IL-2 or TNF-α producing CD8+ T-cells. Of note, the frequencies of CD4+ or CD8+ T-cells producing two (IL-2 + IFN-γ+, TNF-α+IFN-γ+ and IL-2 + TNF-α+) or three (IFN-γ+IL-2+TNF-α+) cytokines were not significantly different (data not shown). These results corroborate those obtained in the IFN-γ ELISpot assay and indicate that the CD4+ T-cell response is predominantly directed to the structural proteins and the CD8+ T-cell response to the non-structural proteins. Furthermore, the frequency of IFN-γ producing CD4+ or CD8+ T-cells correlated inversely with clinical manifestation of meningoencephalomyelitis.

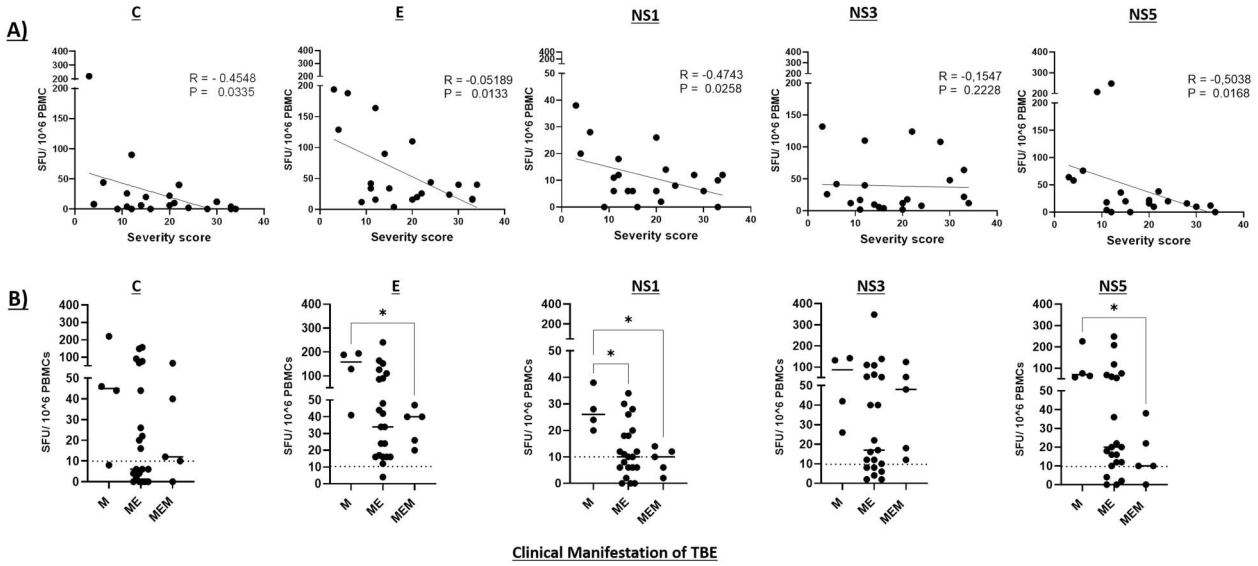

Figure 1. Severity of TBE and clinical manifestations correlate with reduced frequencies of TBEV C-, E-, NS1- and NS5-specific T-cells. (A) Correlation analyses between severity score and IFN-γ spot forming units (SFU) per 1×10^6 PBMCs after stimulation with peptide pools derived from structural or non-structural proteins of TBEV. (B) Frequency of TBEV-specific IFN-γ spot forming units (SFU) per 1×10^6 PBMCs in TBE patients with distinct neurological manifestations (M = meningitis, ME = meningoencephalitis, MEM = meningoencephalomyelitis). The sum of individual values obtained with pools of the TBEV proteins were used to calculate the response to each protein. Each dot represents single study participant and horizontal lines indicate median values. The lines in the correlation analyses represent linear regression. Dashed lines represent cut-off values for responders as described in methods section. A two-tailed Spearman correlation test was employed to calculate *r* and *p* values. Two-tailed Kruskal–Wallis test or One-way Analyses Of Variance (ANOVA) with Dunn's multiple comparison test was performed for comparisons of patients with distinct clinical manifestations. $^*P < .05$.

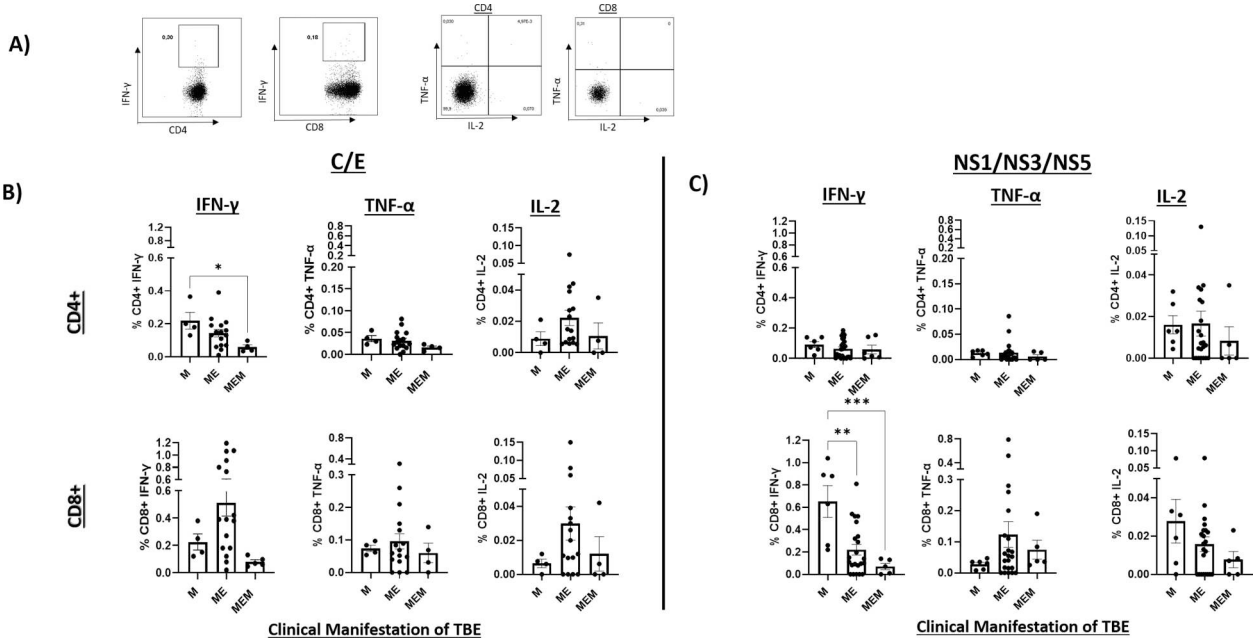

Figure 2. Cytokine expression profiles of CD4+ and CD8+ T-cells specific for structural or non-structural TBEV proteins in patients with meningitis, meningoencephalitis and meningoencephalomyelitis. (A) Representative FACS plots depicting cytokine production by CD4+ or CD8+ T-cells in patients with diverse clinical presentations of TBE. (B) Frequencies of CD4+ (top) and CD8+ (bottom) T-cells producing IFN-γ, TNF-α or IL-2 in response to structural (C or E) proteins of TBEV. (C) Cytokine production by CD4+ (top) or CD8+ (bottom) T-cells towards any of the non-structural proteins of TBEV (NS1, NS3 or NS5) is depicted. Analyses of cytokine response was performed on non-naïve antigen-experienced T-cells. A response above 0.01% was considered positive. Each dot represents T-cell response to any of responding proteins. Horizontal lines indicate median values. Two-tailed Kruskal–Wallis test or ANOVA with Dunn's multiple comparison test were performed for comparisons of groups. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$.

Phenotypic characterization of TBEV-specific T-cells in TBE patients

Next, we analyzed the differentiation stage and phenotypic characteristics of TBEV-specific T-cells. Although the frequency of naïve and effector memory (EM) CD4+ and CD8+ T-cells tended to be lower in meningoencephalomyelitic patients, no statistically significant differences was observed in distribution of the respective CD4+ or CD8+ T-cell subsets in patients with meningitis/meningoencephalitis and those with meningoencephalomyelitis (Figure 3(A) and 3(B)). Next, we investigated the expression of phenotypic markers on non-naïve CD4+ or CD8+ T-cells, like those for activation (CD69, OX40 and HLA-DR), transcriptional regulation (T-box transcription factors T-bet and Eomesodermin), proliferation (Ki-67) and homing (CD29, CD49d or alpha4Integrin1) following peptide stimulation. Although the expression of some of the markers, e.g. OX40, HLA-DR and Ki-67 by both CD4+ and CD8+ T-cells seemed lower in patients with meningoencephalomyelitis, none of these differences reached statistical significance between the three disease manifestations (Figure 3(C)–3(F)).

Analyses of T-cell responses early in the course of TBE and correlation with clinical outcomes one year later

Since the magnitude of the early virus-specific T-cell response inversely correlated with TBE severity, we next addressed the possible correlation with the development of long-term sequelae. All patients who presented with meningitis or meningoencephalitis later developed favorable outcome or some subjective symptoms affecting quality of life (Figure 4(A)). In contrast, all patients with clinical manifestation of meningoencephalomyelitis had objective neurological sequelae one year after acute illness (Figure 4(A)). Consistent with the significantly higher E-, NS1- and NS5-specific T-cell responses observed in patients with the relatively mild forms of TBE, patients with strongest TBEV E, NS1 and NS5-specific T-cell responses early after diagnosis of TBE, also had a favorable long-term outcome without neurological sequelae (Figure 4(B)). However, when clinical manifestation of TBE was not considered, statistically significant differences could not be demonstrated in the magnitude of the T-cell response between the groups with different long-term outcomes of TBE (Figure 4(C)).

TBEV-specific antibody responses in TBE patients with different clinical manifestations and disease severities and correlations with long-term sequelae

Animal models have demonstrated that virus-specific antibodies contribute to protective immunity against TBEV infections [13,17–21]. Also in humans, TBEV-specific antibodies have been identified as a correlate of protection [6]. In the latter study, low or undetectable virus-specific IgG antibody levels early in the course of TBE, correlated with disease severity and unfavorable long-term disease outcomes [6]. In the present study, we extended analysis of the antibody responses and tested TBEV-specific IgG and IgM antibodies, VN antibody titers (VNT_{100}), TBEV E-domain III (EDIII)- and NS1-specific antibodies in serum samples of subjects of which also T-cell data were obtained. In these subjects, IgG antibody levels together with IgM, VNT_{100}, EDIII- and NS1-specific antibody levels did not differ between TBEV-infected patients with distinct clinical manifestations or disease severities (Figures 5(A) and 5(B)). Furthermore, no correlation between the antibody responses and disease outcome one year after acute illness was observed (Figure 5(C)).

Discussion

In the present study, we have shown that differential TBEV-specific T-cell responses early in the course of TBE correlate with clinical manifestation and disease severity of acute illness. More specifically, poor T-cell responses to structural and some non-structural proteins correlated with severity scores and development of TBE meningoencephalomyelitis. Patients with meningoencephalomyelitis also had a poor long-term outcome of TBE, with objective neurological sequelae. Differential antibody responses early after infection did not correlate with severity and outcome of TBEV infection. To our knowledge, this is the first study that provides in-depth information on TBEV-specific immune responses and their correlation with disease severity and adverse clinical manifestation.

Various studies have identified clinical and sociodemographic parameters associated with severity and long-term outcome of TBE, like monophasic TBE presentation, co-presence of Lyme neuroborreliosis, diabetes, previous TBE vaccination, increased age, male gender, increased serum CRP concentration and increased CSF cell count [17,22–24]. However, virus-specific immune responses induced early after TBEV infection and their possible correlation with severity of acute illness and incomplete recovery have received limited attention. Particularly, in-depth analyses of TBEV-specific T-cell responses early after TBE diagnosis would be of interest to obtain a better understanding of the mechanisms of viral clearance and potentially immune-mediated pathogenesis of TBEV infections, and may provide predictive value for disease outcome. In the present study, we tested the magnitude, specificity and functional properties of TBEV-specific T-cells early after diagnosis of mild and severe TBE. Patients with

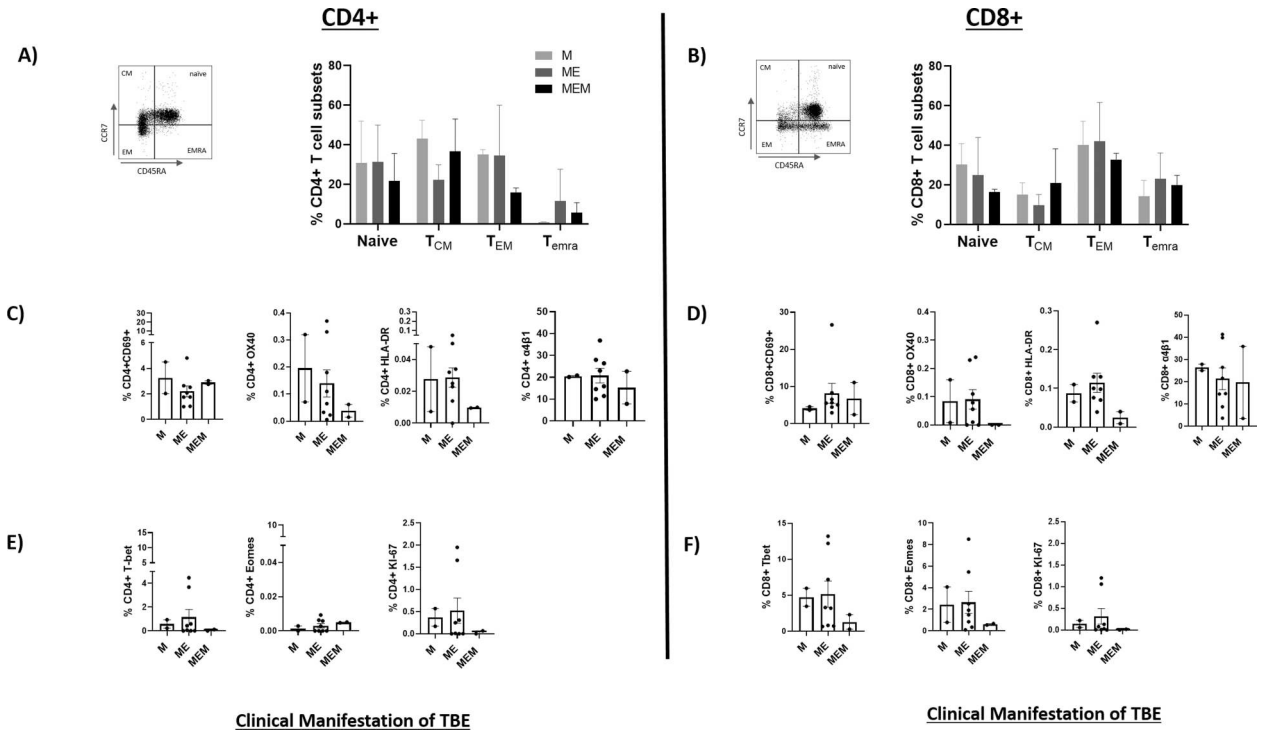

Figure 3. Phenotypic characterization of TBEV-specific T-cells in patients with distinct clinical manifestations. Representative FACS plots (left) and summary distribution of T-cell subsets (right) of CD4+ **(A)** or CD8+ **(B)** T-cells in patients with mild or severe clinical presentation of TBE (without stimulation). Expression of activation markers (CD69, OX-40 or HLA-DR) or homing marker (α4β1) by CD4+ **(C)** or CD8+ **(D)** T-cells after stimulation with combination of highest responding peptide pools representing either structural or non-structural proteins. Intranuclear detection of T-bet, Eomes or Ki-67 by CD4+ **(E)** or CD8+ **(F)** T-cells upon stimulation similar to above. Only samples with sufficient cell numbers after the functional cytokine assays were used and values from unstimulated controls were subtracted for the analyses of the indicated phenotypic and intranuclear markers. T_{naive} = naïve T cell, T_{CM} = central memory T cell, T_{EM} = effector memory T cell, T_{EMRA} = terminally differentiated effector T cell. Two-tailed Kruskal–Wallis test or ANOVA with Dunn's multiple comparison test were performed for comparisons of groups.

meningoencephalomyelitis, a severe clinical manifestation of TBE, displayed significantly lower E-, NS1- and NS5-specific T-cell responses than patients with relatively mild meningitis. The inverse correlation between magnitude of virus-specific T-cell responses directed to structural and some non-structural proteins with disease severity was also observed when disease severity scores were used. These findings support the hypothesis that effective induction of TBEV-specific T-cells early after TBEV infection affords protection against the development of severe disease, potentially by blunting virus replication and accelerating viral clearance by cytolytic activity or secretion of cytokines with antiviral activity (9). During the peak of the T-cell response, effector memory T-cells expressing Eomes + Ki-67 + T-bet + dominated but transitioned into other T-cell memory subsets as infection resolved [12,25]. We also examined whether the activation stage and subsets of CD4+ or CD8+ T-cells correlated with disease severity. Although the frequency of naïve and effector memory CD4+ and CD8+ T-cells tended to be lower in TBE patients with meningoencephalomyelitis, these differences were not statistically significant. Likewise, the expression of activation markers (CD69, OX40 and HLA-DR) by both CD4+ or CD8+ T-cells and transcriptional and proliferative markers (T-bet, Eomes and Ki-67) did not differ significantly in patients with mild or severe manifestations of TBE. Apart from affording protection, TBEV-specific T-cells might contribute to neuropathology through targeting infected neurons as has been described previously for TBEV and other flaviviruses [1,26–30]. T-cells that acquire the capacity to migrate into the central nervous system were shown to express α4β1 during the neuroinvasive stage of TBE [25]. Therefore, we investigated if α4β1 was differentially expressed by CD4+ or CD8+ T-cells in patients with meningitis or meningoencephalomyelitis, which was not the case. The frequencies of these cells may have been too low or we may have missed the kinetics of cells circulating in the blood. Thus, none of the markers for T-cell activation, differentiation, transcriptional activity and homing of TBEV-specific T-cells correlated with disease severity early after diagnosis. Of note, virus-specific CD4+ T-cells were mainly directed towards structural proteins of TBEV, whereas the CD8+ T-cells responded to the non-structural proteins, which is in accordance with previous studies that investigated the specificity of CD4+ and CD8+ T-cells against TBEV and Japanese encephalitis virus [12,16,31].

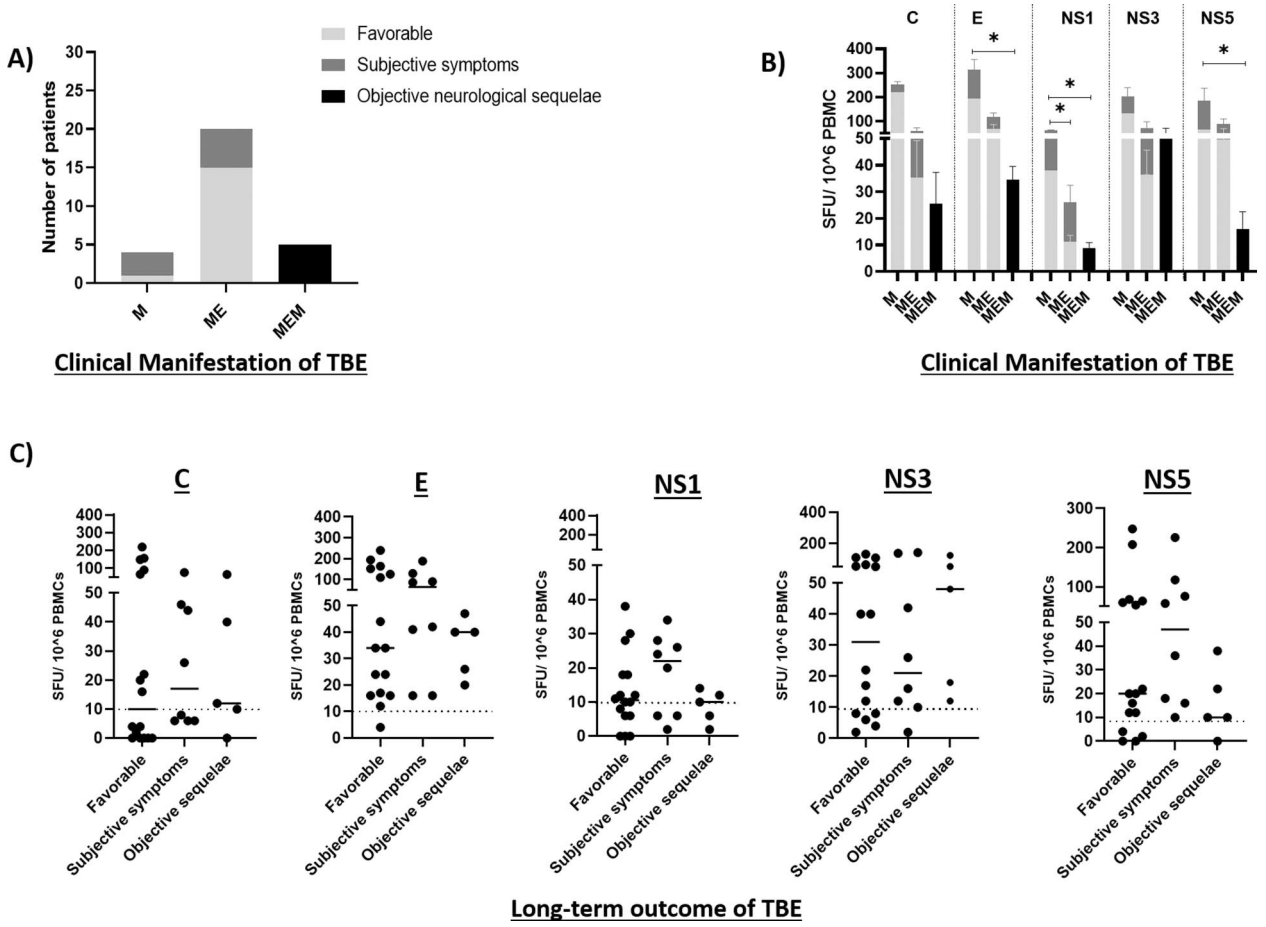

Figure 4. Correlation of T-cell responses during early TBE and clinical outcomes one year later. (A) Number of patients with distinct clinical manifestations early during TBE that later developed favorable (light grey), subjective symptoms affecting quality of life (grey) or objective neurological sequelae (black) one year after acute illness. (B) Frequency of TBEV-specific IFN-γ producing T-cells per 1×10^6 PBMCs in patients with distinct clinical manifestations during early TBE and outcome one year later. (C) Frequency of TBEV-specific IFN-γ producing T-cells per 1×10^6 PBMCs during acute illness (without categorizing based on clinical manifestations) in patients that latter had distinct clinical outcomes. Bars represent mean plus standard errors of the mean and each dot represents a single patient. A two-tailed Kruskal–Wallis test or ANOVA with Dunn's multiple comparison test were performed for comparisons of groups. *$P < .05$.

Previous studies showed that poor virus-neutralizing antibody responses are associated with severe disease and clinical outcome of TBE [6,7]. Upon infection with TBEV, stronger antibody responses to the E and NS1 proteins are induced than after vaccination [2,32]. We recently showed in mice that antibodies against TBEV E and NS1 proteins afford full or partial protection [13,18]. In the present study, we determined virus-specific IgG, IgM levels and antibody responses to the EDIII and NS1 protein and their neutralizing activity in view of disease severity and outcome. TBEV-specific antibody titers early after TBEV infection did not differ between patients with mild or severe TBE, which is in contrast with previous studies [6]. This may be due to the smaller number of patients of which matching serum and PBMC were available in the present study, which allowed us to test virus-specific antibody and T cell responses in parallel.

All patients who presented with meningoencephalomyelitis early after diagnosis of TBE and who had poor virus-specific T-cell responses, developed post-encephalitis syndrome with objective neurological sequelae one year later, which to some extent is in concordance with previous findings [7]. In contrast, none of the patients that presented with meningitis or meningoencephalitis during acute illness developed objective neurological sequelae. Thus, patients who had mild TBE and favorable long-term clinical outcome also had the highest frequencies of TBEV-specific IFN-γ producing T-cells suggesting that robust induction of virus-specific T-cells early after TBEV infection affords protection against disease severity and possibly also against long-term neurological sequelae. However, without stratifying for disease severity, the magnitude of the virus-specific T-cell response did not correlate with the long-term clinical outcome, indicating its multifactorial nature.

This study has its limitations and strengths. The number of patients of which PBMC and serum was available to study both T-cell and antibody responses, was relatively small. However, the opportunity to

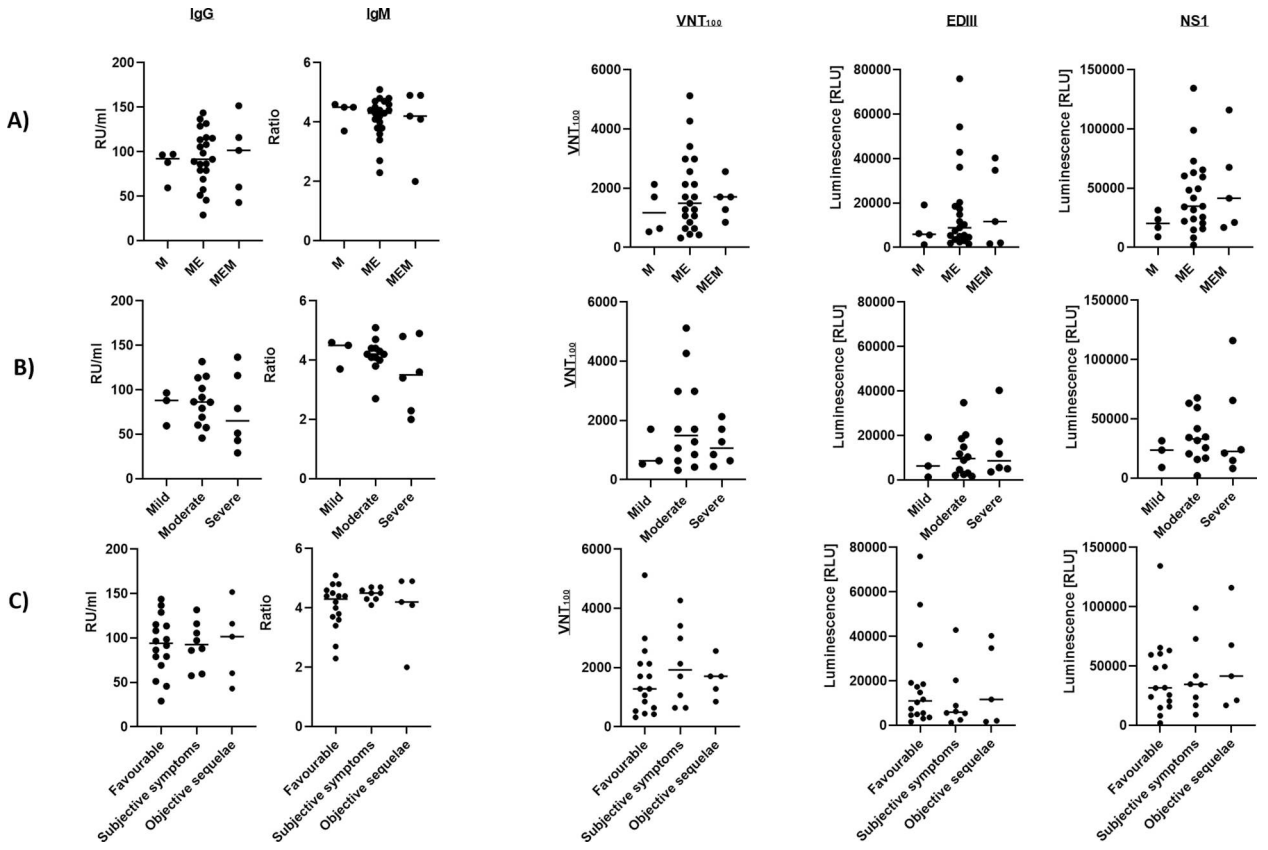

Figure 5. TBEV-specific antibody responses in patients with different clinical manifestations and disease severity of TBE and correlations with long-term sequelae. (A) Titers of TBEV Neudoerfl-specific IgG, IgM, EDIII, NS1 and virus-neutralizing titers (VNT_{100}) in serum samples obtained early in the course of TBE in patients with clinical manifestation of meningitis, meningoencephalitis or meningoencephalomyelitis. (B) VNT_{100}, IgG, IgM, EDIII- and NS1-specific serum antibody titers in TBE patients with indicated severity scores. (C) Analyses of serum antibody titers of patients with distinct long-term sequelae. In all cases, horizontal lines indicate mean with standard errors of the mean. ANOVA with Dunn's multiple comparison test was performed for comparisons of groups.

examine immunological responses in detail and to correlate those with the early disease course and long-term outcome of TBEV infection is unique. Only patients infected with the European subtype of TBEV were studied and the outcome of infections with the other subtypes may be different or even may offer a bigger window of opportunity to observe differences in patients with strong or poor immune responses early after TBE diagnosis. It would be of interest to perform prospective studies, which would allow correlating immune responses with viral loads. The patients included in the present study had at admission neurological symptoms, which present at later stage of the infection at which viral loads are declining or at which the virus even has been cleared, precluding the assessment of viral loads reliably. It would also be of interest to investigate the correlation between immunological outcome of (early) infection and biomarkers of brain damage as described recently [33]. TBEV infected patients without neurological symptoms could not be included in this study, because these patients do not present themselves and often remain unnoticed.

In summary, our data indicate that poor TBEV-specific T-cell responses early after diagnosis of TBE correlate with severe acute illness, including the development of paresis (meningoencephalomyelitis). We speculate that poor T-cell responses upon TBEV infection are the cause of more severe disease and clinical manifestation, rather than the consequence, but further studies are required to confirm this. If this is the case, improved TBE vaccines not only should aim at the induction of virus-neutralizing antibodies but also at the induction of T-cell responses to structural and non-structural proteins.

Author contributions

Conceptualization: AO, GR, AA and TAZ. Funding acquisition: AO, GR, IS and TAZ. AA and JS performed most of the experiments, analysed the data and wrote the draft manuscript. AA, JS and IS performed the LIPS assay. MKu, JB and AA performed the virus-neutralization assay. FS and PB recruited patients and collected samples. MKo, KRR, NK, and JS isolated PBMC and sera samples. Writing, review and editing: AA, JS, PB, MKo, KRR, NK, MKu, JB, GS, IS, FS, TAZ, AO, GR. All authors have read and commented on the manuscript.

Disclosure statement

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