Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Intrathecal expression of IL-5 and humoral response in patients with tick-borne encephalitis

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ARTICLE INFO

Keywords:
Tick-borne encephalitis
Pleocytosis
Serologic response
B-lymphocytes
IL-5

ABSTRACT

Aim: The aim of the study was to assess the role of an early specific humoral response in human infection with a tick-borne encephalitis virus (TBEV) and the role of IL-5 as its potential mediator and marker.
Materials and methods: The retrospective study involved a cohort of 199 patients diagnosed with TBE, in whom anti-TBEV IgM and IgG antibody titers were analyzed on admission and compared with clinical presentation and basic laboratory parameters. The prospective study included 50 TBE patients in whom IL-5 serum and CSF concentration was measured with ELISA on admission in the TBE neurologic phase and in selected patients before discharge, at follow-up or in samples obtained before the neurologic phase onset.
Results: The serum anti-TBEV IgM correlated with good clinical outcome and the CSF anti-TBEV IgM with more pronounced CSF inflammation on admission, but also with its more complete resolution on follow-up. The serum anti-TBEV IgG correlated with milder presentation and better outcome. Concentration of IL-5 was increased in CSF but not in the serum of TBE patients. IL-5 concentration index on admission favored its intrathecal synthesis. IL-5 did not correlate significantly with clinical presentation and specific IgM and IgG titers.
Conclusions: Specific anti-TBEV IgM systemic and intrathecal response and IgG systemic response are protective, together favoring milder presentation, better outcome and resolution of central nervous system (CNS) inflammation. IL-5 is expressed intrathecally in TBE, but its pathogenetic role remains unclear.

1. Introduction

Tick-borne encephalitis (TBE) is an acute infectious disease involving central nervous system (CNS), caused by a zoonotic flavivirus (tick-borne encephalitis virus, TBEV) which is transmitted by *Ixodes* ticks. It is endemic in the temperate zone of Asia, Eastern and Central Europe, where several thousand cases are reported annually (Mansfield et al., 2009; Randolph, 2001). Infection with the European sub-type of TBEV may be asymptomatic or result in a mild, flu-like disease (Gustafson et al., 1992). If the virus penetrates into CNS, the disease may take form of uncomplicated meningitis or neurologic involvement (meningoencephalitis or meningoencephalomyelitis) of variable severity, from relatively mild to life-threatening and/or causing permanent neurologic deficits (Czupryna et al., 2011; Kaiser, 2002; Mickiené et al., 2002; Schellinger et al., 2000). The pathogenesis of nervous tissue damage is complex and seems to result from a combination of the TBEV direct effect on neurons and local inflammatory response. The neural cells are highly susceptible to TBEV infection and when infected die either by apoptosis or necrosis (Růžek et al., 2009) while TBEV-infected astrocytes become a potent source of pro-inflammatory mediators, with possible pathologic consequences to the nervous tissue (Palus et al., 2014). Animal models and clinical studies involving TBE patients show a prevalent intrathecal cellular response including Th1 CD4+ lymphocytes and cytotoxic CD8+ lymphocytes, likely involved in the disease immunopathogenesis and neuronal damage (Gelpi et al., 2006; Holub et al., 2002; Jeren and Vince, 1998; Růžek et al., 2009). Although B lymphocytes are scarce in CSF compared to T cells (Jeren and Vince, 1998), data suggest that a peripheral and intrathecal humoral response to TBEV may play an important protective role. Serologic IgM response is early and highly specific in different flavivirus infections, for example in dengue its onset coincides with the defervescence and the end of viremia (Innis et al., 1989). Analogously, IgM and IgG antibodies are usually detectable in serum at the onset of the neurologic phase of TBE, while in CSF specific IgM can be detected in about 50% of patients early in the neurologic phase and in practically all 10 days after the first symptoms of CNS involvement (Holzmann, 2003). In a study by Günther et al. (1997) specific IgM was detectable in serum at the beginning of the neurologic phase and peaked in CSF in

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https://doi.org/10.1016/j.ttbdis.2018.03.012
Received 19 November 2017; Received in revised form 10 March 2018; Accepted 11 March 2018

the second week after the onset with the intrathecal production documented in >90% of patients. An elevated IgG index pointing to a high intrathecal immunoglobulin synthesis often persists into the convalescent period of the disease (Günther et al., 1997). In CNS infections with other flaviviruses the serologic response may be indispensable for the control of the infection, as shown in a mouse model of West Nile virus (WNV) infection and in patients with Japanese encephalitis (JE) (Diamond et al., 2003a,b; Libraty et al., 2002). The studies on TBE patients suggest the protective role of the early serologic response in periphery (Atrasheuskaya et al., 2003; Günther et al., 1996; Günther et al., 1997; Kaiser and Holzmann, 2000; Toporkova et al., 2008), while the significance of the intrathecal synthesis of the specific antibodies is less clear, as their high titer and prolonged presence tend to associate with more serious neurologic involvement (Günther et al., 1997; Kaiser, 2002). It may be suspected that the delayed and/or originally ineffective intrathecal serologic response contributes to more severe neurologic phase, with worse control of the infection and predominant immunopathogenic cellular response, but more data on the association of the serologic response with the clinical presentation and outcome are needed to confirm that hypothesis.

The role of intrathecal B lymphocytes presence and activation status has not been directly studied in TBE so far. These cells constitute only up to a few percent of all CSF lymphoid cells and are scarce in the CNS infiltrates in flavivirus encephalitis, but their association with the clinical manifestation and outcome is not known (Grygorczuk et al., 2016; Holub et al., 2002; Jeren and Vince, 1998). CXCL13, chemokine acting predominantly on B lymphocytes, seems to play an important role in the pathogenesis of acute autoimmune and enteroviral encephalitis (Kothur et al., 2016) and is characteristically up-regulated in CSF of patients with neuroborreliosis. However, although detected as increased in serum and CSF of patients with TBE, the increase of its concentration is relatively modest and does not form a clear chemotactic gradient toward CSF, making its role as a marker of B cell-dependent response dubious in this setting (Cerar et al., 2013; Pietikäinen et al., 2016; Zajkowska et al., 2011). Here we have attempted to study another cytokine involved in B cell-dependent immunity, IL-5. In humans IL-5 plays an important role in the immune response, inflammation and disease control, including, although not limited to, specific humoral responses (Takatsu, 2011). IL-5 is a factor that induces terminal differentiation of B cells to Ig-secreting cells. Stimulation of Il-5 induces rapid tyrosine phosphorylation of cellular proteins including the βc, SH2/SH3-containing proteins such as Vav and Shc, Btk and Btk-associated molecules, JAK1/JAK2 and STAT1/STAT5, PI3K and MAP kinases that activate downstream signaling molecules (Horikawa et al., 2001; Huang et al., 2006; Kagami et al., 2000; Kouro et al., 1996; Martinez-Moczygemba et al., 2007; Meads et al., 2010; Ogata et al., 1998; Satoh et al., 1995; Takaki et al., 1994; Takatsu, 2011; Zahn et al., 2000). Il-5 induces CD38-activated splenic B cells to differentiate into immunoglobulin M-secreting cells and undergo μ to γ1 class switch recombination (CSR) at the DNA level, resulting in immunoglobulin G1 (IgG1) production (Horikawa et al., 2001). Its intrathecal expression in CNS infections and role in their pathogenesis remains largely unknown.

The aim of our study was to assess the role of IL-5 expression and early serologic response in the pathogenesis of TBE. Therefore, we retrospectively studied the relation between the serologic response and selected clinical and laboratory parameters in a large group of patients with TBE. Following that, expression of IL-5 was evaluated prospectively in a smaller group of selected patients.

2. Materials and methods

2.1. Retrospective study

2.1.1. Patients

Clinical registry of diagnoses of patients hospitalized in the Department of Infectious Diseases and Neuroinfections of the Medical University of Białystok, Poland, from September 2010 to December 2014 was screened for patients with the established diagnosis of TBE with the revision of their clinical and laboratory data. We identified 243 patients, of whom 20 were excluded because of the uncertain diagnosis (serologic testing towards TBE not performed or all the available results negative), leaving 223 patients with a documented specific anti-TBEV IgM response in at least one serum or CSF sample (on admission or seroconversion in follow-up samples). Further 24 patients in whom the initial serologic examination was not performed and the diagnosis was based solely on the follow-up serology were excluded. The final study group consisted of 199 patients.

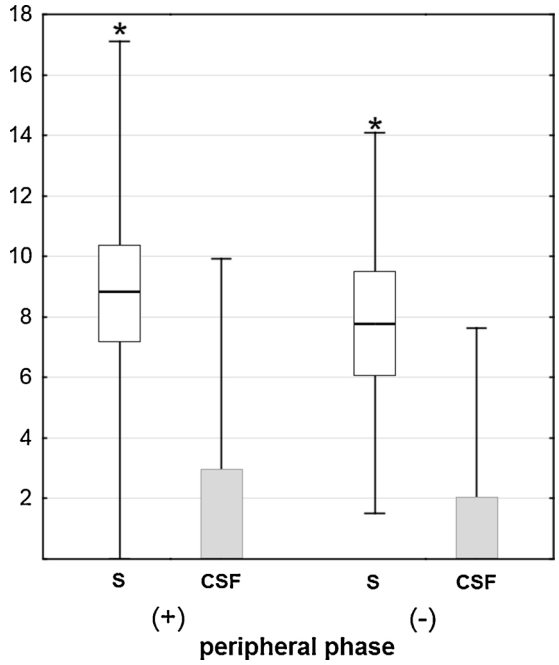

Fig 1. The specific anti-TBEV IgM score in serum (S, empty bars) and cerebrospinal fluid (CSF, light grey bars) on admission to hospital in patients with TBE with typical biphasic (+); (n = 85) and monophasic (−); (n = 93) clinical presentation. Shown are the median (horizontal line), quartiles (box) and maximum values (whiskers). * − the significant difference with $p < 0.05$.

2.1.2. Procedures

The time from symptoms onset, the course of the disease at home, as well as the clinical presentation and severity on admission and complications during hospitalization, the outcome on discharge and during follow-up visits, and the results of laboratory examinations were extracted from patients' records. The disease severity was scored from 0 to 6 using an arbitrary pre-defined scale designed for the purpose of the study, in which 0 corresponded to a flu-like infection, 1 to an uncomplicated meningitis, 2 − mild CNS involvement with no altered mental status nor paresis (limited, transient neurologic symptoms, e.g. Babinski sign, mild ataxia, paresthesia, tremor), 3 − relatively mild encephalitis with lethargy, drowsiness, altered affect, mild monofocal paresis, gait disorders, but patients in logical contact and able to walk; 4 − moderately severe encephalitis with disorientation, multifocal and/or severe focal neurologic symptoms, generalized seizures; 5 − loss of consciousness; 6 − coma or death. Patients were also stratified according to the course of the disease (typical biphasic with a distinct initial flu-like peripheral phase or monophasic), presentation in the neurologic phase (meningitis, meningoencephalitis, meningoencephalomyelitis), abnormalities in mental status (defined as mild − slowliness, lethargy; moderate − disorientation, agitation, psychotic symptoms; severe − lack of any logical contact or loss of consciousness), presence of paresis, as well as outcome on discharge from hospital or during planned follow-up visits within weeks after discharge (defined