Cytokine 71 (2015) 125–131

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Increased concentration of interferon lambda-3, interferon beta and interleukin-10 in the cerebrospinal fluid of patients with tick-borne encephalitis

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ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 1 October 2014
Accepted 4 October 2014
Available online 29 October 2014

Keywords:
Tick-borne encephalitis
IL-10
IFNλ3
IFNβ

ABSTRACT

Tick-borne encephalitis (TBE) has a wide clinical spectrum, from asymptomatic to severe encephalitis, and host-dependent factors determining the outcome remain elusive. We have measured concentrations of pro-inflammatory/Th1 interferon-γ (IFNγ), immunomodulatory/Th2 interleukin-10 (IL-10), anti-viral type I (IFNβ) and type III (IFNλ3) interferons in cerebrospinal fluid (csf) and serum of 18 TBE patients, simultaneously genotyped for polymorphisms associated with the expression of genes *IFNL3* (coding IFNλ3), *IL10*, *CD209* and *CCR5*. IL-10, IFNβ and IFNλ3 were up-regulated in csf, with IFNλ3 level higher in patients with the milder clinical presentation (meningitis) than in meningoencephalitis. There was an increased serum IFNβ and a tendency for increased serum IL-10 in meningitis patients. Genotype in rs12979860 locus upstream of *IFNL3* was associated with IFNλ3 expression and in rs287886 (*CD209*) – IL-10 expression. IL-10, IFNβ and IFNλ3 are expressed and play a protective role in TBE and their expression in TBE patients is associated with genetic polymorphisms.

1. Introduction

Tick-borne encephalitis (TBE) is caused by a tick-borne encephalitis virus (TBEV, *Flavivirus* genus, Flaviviridae) endemic in most of the temperate zone of Eurasia [1]. European TBEV subtype may cause asymptomatic infection, flu-like disease or biphasic disease with the central nervous system (cns) involvement in the second phase. The later presents as meningitis, meningoencephalitis or meningoencephalomyelitis of different severity, from self-limited to life-threatening [1,2]. Reasons of this clinical diversity are not fully understood, but host-related factors, including genetically determined variability of the inflammatory/immune response, are likely to play an important role [3–7]. The outcome of a neurotropic *Flavivirus* infection is decided at several stages, including (1) the initial response at the entry site; (2) the extent of the peripheral infection and related inflammation; (3) the penetration into cns (neuroinvasiveness); (4) the potential to cause neuronal damage (neurovirulence), with complex interactions between the virus and the host response at each of these steps [8–10]. The cns entry is facilitated by a high viremia, but also by a systemic inflammation impairing the integrity of the blood/csf barrier [10] and the neuronal damage depends both on the virus replication and on the secondary immunopathology [8,11–13]. The host response to TBEV involves Th1-type inflammation, a Th2/humoral response and Tc-dependent cytotoxicity, with the role of particular elements in the virus clearance and cns pathology still debatable [11–13].

In the current study we have attempted to evaluate the role of four cytokines in human TBEV infection: Th2/immunomodulatory interleukin 10 (IL-10) [14], Th1/pro-inflammatory interferon γ (IFNγ) [15,16] and two members of the interferon family involved in the anti-viral response: IFNβ and IFNλ3, as well as the potential association of their expression with the genetic background. IL-10 and IFNγ have been previously detected in body fluids of TBE patients and may act as antagonists, with IL-10 related to the protective serologic response and immunomodulation and IFNγ to the intrathecal inflammation and immune-mediated tissue damage

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http://dx.doi.org/10.1016/j.cyto.2014.10.001
1043-4666/

[3,13,17,18]. Type I interferons (including IFNα and IFNβ) are involved in anti-viral defense and up-regulated by the recognition of viral molecular patterns, including binding of a double-stranded RNA by Toll-like receptor 3 (TLR3). They act in an autocrine and paracrine manner up-regulating the expression of hundreds of IFN-stimulated genes (ISGs), eventually boosting the cell anti-viral defense mechanisms [19,20]. Type III interferons: IFNλ1 (IL-29), IFNλ2 (IL-28A), IFNλ3 (IL-28B) and IFNλ4 act similarly to type I IFNs, but through a distinct receptor restricted to fewer cell populations, for example B lymphocytes among the peripheral blood mononuclear cells [21–24]. This selectivity may enable them to cause a qualitatively distinct response *in vivo*, and both classes of IFNs cooperate to achieve effective anti-viral protection [21,25]. According to Dellgren et al. *in vitro* study IFNλ3 may be the most biologically active member of the family followed by IFNλ1 [22]. The recently identified IFNλ4 is expressed only in the presence of the genetic variant ΔG in ss469415590 locus upstream of the gene for IFNλ3, and its role and biologic activity is still not fully understood [26,27]. The *in vitro* and animal data suggest a protective role of type I IFNs against neurotropic *Flavivirus* [16,28,29], including West Nile virus (WNV) related to TBEV and responsible for a disease resembling TBE both in its clinical presentation and in its variable severity [30]. Little is known about the role of IFNλ in *Flavivirus* infections, but *in vitro* IFNλ3 and IFNα have a synergistic and complementary effect on the replication of another member of Flaviviridae, hepatitis C virus (HCV) [25].

Polymorphisms in genes involved in an anti-viral/inflammatory response, including *CCR5Δ32* deletion in the gene for the chemokine receptor CCR5 and single nucleotide polymorphisms (SNPs) in the genes for TLR3 and CD209, has been proven to correlate with the risk and course of TBE. However, each of them plays only a minor role individually, making the involvement of additional genetic factors likely [4–7]. Of these, the low-producing *IL-10* genotype (rs1800896 AA) increased the risk of symptomatic TBE in the Swedish population, but the trend was not statistically significant and the finding remains unconfirmed [4]. SNPs related to the *IFNL3* gene coding IFNλ3 have a prominent impact on the outcome of HCV infection [31,32] and may represent another level of a genetic variability associated with the susceptibility to TBEV, potentially more important than previously described SNPs.

The better understanding of the host response could elucidate the natural variability of the TBEV infection, making it possible to identify genotypic or phenotypic risk factors of a symptomatic disease and of neurologic complications, and eventually to individualize prophylaxis and treatment options dependent on the individual risk. In this study, we have investigated (1) the expression of IL-10, IFNγ and selected type I (IFNβ) and III (IFNλ3) interferons in TBE; (2) its correlation with the clinical severity and laboratory markers of the peripheral and intrathecal inflammation; (3) the dependence of IFNλ3 and IL-10 expression on the SNPs associated with *IFNL3* and *IL-10* genes; (4) the relation between the *CCR5Δ32* mutation and SNPs in *CD209* gene and the expression of the studied cytokines.

2. Material and methods

The study group comprised eighteen patients of the Department of Infectious Diseases and Neuroinfections of the Medial University in Białystok, with a clinical diagnosis of TBE confirmed serologically with Enzygnost Anti-TBE/FSME Virus assay (Siemens, Munich, Germany) (Table 1). Meningitis was diagnosed in 4 patients with normal mental status and no neurologic abnormalities, mild meningoencephalitis in 7 with pathologic reflexes or other isolated neurologic abnormalities (tremor, gait disturbances, paresthesia) and moderately severe meningoencephalitis in 6 with impaired consciousness or multiple/severe neurologic deficits, including paresis. A patient with meningoencephalomyelitis with a flaccid brachial paresis was included in the moderately severe meningoencephalitis group for analysis. No patient presented with loss of consciousness, multifocal paresis or life-threatening complications. Control sera ($n = 8$) were obtained from 8 healthy blood donors and control csf ($n = 8$) from patients in whom meningitis was excluded by a lumbar puncture. The study was approved by the Ethics Committee of the Medical University in Białystok. A written informed consent was given by participants.

The blood (into EDTA containing tubes) and csf were obtained during diagnostic procedures: on admission early in the neurologic phase and in the convalescence period 12–16 days later, stored at −80 °C and thawed before testing. Inflammatory parameters in

Table 1
Patients with tick-borne encephalitis (TBE) included in the study.

No	Age	Sex	Clinical form	csf Parameters on admission				Genotyping performed
				Cell count[a]	Lymphocytes[a]	Protein[b]	Albumin[c]	
1	44	Male	M	98	54	0.44	0.32	Yes
2	39	Male	M	112	76	0.63	0.46	Yes
3	21	Male	M	228	160	0.81	0.58	Yes
4	63	Female	M	59	15	0.59	0.41	Yes
5	26	Male	ME, mild	134	105	0.81	0.56	Yes
6	49	Female	ME, mild	83	71	0.52	0.35	Yes
7	49	Female	ME, mild	75	50	0.48	0.40	Yes
8	42	Female	ME, mild	61	21	0.37	0.23	Yes
9	54	Male	ME, mild	99	33	0.70	0.41	Yes
10	54	Female	ME, mild	127	99	0.53	0.37	Yes
11	24	Female	ME, mild	40	27	0.46	0.33	No
12	59	Male	ME, moderate	138	36	0.77	0.73	Yes
13	79	Female	ME, moderate	40	20	0.35	0.21	Yes
14	57	Male	ME, moderate	170	129	0.76	0.52	Yes
15	67	Male	ME, moderate	41	18	0.64	NA	No
16	50	Male	ME, moderate	115	99	1.24	0.97	No
17	86	Male	ME, moderate	44	25	0.59	0.41	Yes
18	44	Male	MEM	171	84	0.97	0.71	Yes

M – meningitis, ME – meningoencephalitis (mild or moderately severe), MEM – meningoencephalomyelitis.
[a] cells per μL.
[b] g/L (normal 0.3–0.45).
[c] g/L (normal 0.1–0.3).