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Expression of the interleukin 17 in cortical tubers of the tuberous sclerosis complex

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ABSTRACT

The role of interleukin 17 (IL-17) to epilepsy-associated cortical tubers of tuberous sclerosis complex (TSC) is unknown. We investigated the expression patterns of the IL-17 and IL-17 receptor (IL-17R) in cortical tubers of TSC compared with normal control cortex (CTX). We found that IL-17 and IL-17R were clearly upregulated in cortical tubers at the protein levels. Immunostaining indicated that IL-17 was specifically distributed in the innate immunity cells (DNs, GCs, astrocytes, and microglia) and adaptive immunity cells (T-lymphocytes) as well as the endothelial cells of blood vessels. The overexpression and distribution patterns of IL-17 may be involved in the epileptogenicity of cortical tubers in TSC.

1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder resulting from mutations in one of two genes, TSC1 (which encodes hamartin) and TSC2 (which encodes tuberin), and is characterized by hamartoma formation in multiple organs, including the skin, eyes, kidney, heart, and brain(Crino et al., 2006). Neurological involvement usually accounts for the most disabling symptoms of the disease, such as intellectual disabilities, autism, and epilepsy. Epilepsy has a high prevalence in TSC, occurring in more than 70% to 80% of patients (Crino et al., 2006; Curatolo et al., 2008). Patients with TSC can have multiple seizure types (including generalized, focal, or multifocal seizures) and are refractory to the currently available antiepileptic drugs (AEDs) (Crino et al., 2006). Histopathological examination of TSC brain specimens reveals cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas (SEGAs). Cortical tubers, a typical pathological hallmark of TSC in the brain, are regions of focal cortical dysplasia presenting with disorganization or lacking of the normal six-layered cortical lamination structure, astrogliosis, abnormal cells including dysplastic neurons (DNs), with aberrant somatodendritic morphologies, and giant cells (GCs, also named TS-cells) with short thickened processes (Mizuguchi and Takashima, 2001). Moreover, cortical tubers are often identified as source of seizures and require surgical therapy (Luat et al., 2007). An understanding of the molecular events that underlie the occurrence of seizures is essential for devising new therapeutic approaches for the treatment of epilepsy.

The expression of proinflammatory cytokines is upregulated in cortical tubers of TSC, and plays a critical role in generating seizures (Boer et al., 2008, 2010). IL-17, also referred to as IL-17A, is a prototypic member of the newest subclass of cytokines. IL-17 acts as a proinflammatory cytokine that can induce the release of certain chemokines and cytokines (e.g., IL-1β, IL-6, TNF-α) and has a distinct ligand–receptor system (IL-17R) (Xu and Cao, 2010). Several studies have demonstrated that IL-17 can play a bridging role between innate and adaptive immunity in vivo and that IL-17 induces blood–brain barrier (BBB) disruption and promotes neuronal injury through an IL-17/IL-17R combination in multiple sclerosis and ischemic brain injury (Kolls and Linden, 2004; Kebir et al., 2007; Wang et al., 2009; Xu and Cao, 2010). Activation of both the innate and adaptive immune responses has been accepted as a striking feature that occurs in epilepsy-associated cortical tuber and that the inflammatory response may contribute to the generation and recurrence of seizures (Boer et al., 2008; Rodgers et al., 2009). Recent evidence suggests that BBB disruption is associated with inflammation in TSC-associated lesions, which facilitates neuronal hyperexcitability and epileptiform activity (Boer et al., 2008). There is activation of the mammalian target of rapamycin (mTOR) signaling pathway in TSC. Interestingly, rapamycin, a potent specific inhibitor of the mTOR signaling system, strongly inhibits the induction of IL-17 in T lymphocytes (Yurchenko et al., 2012) which was observed in cortical tubers (Boer et al., 2008). In addition, our previous study revealed that there are increased levels of IL-17 and IL-17R protein in cortical lesions of focal cortical dysplasias (FCDs) (He et al., 2013), which are recognized to be causes of pediatric intractable epilepsy. Moreover, FCDIIb and TSC do share a number of characteristic cellular and histological abnormalities as well as common clinical features. Accordingly, we hypothesize that

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IL-17 may play a key role in the epileptogenesis and may represent a potential anti-epileptogenic target. Therefore, the identification of IL-17 and IL-17R in the epileptic foci would provide a new basis for TSC epileptogenicity.

In the present study, we analyzed the protein levels of IL-17 and IL-17R in cortical tubers from patients with medically intractable epilepsy. In addition, we investigated the specific cellular distribution of IL-17 and IL-17R in this lesion.

2. Materials and methods

2.1. Subjects

The cases in this study were obtained from the Department of Neurosurgery of the Xinqiao Hospital (Third Military Medical University, Chongqing, China). All of the procedures and experiments were conducted under the guidelines approved by the Ethics Committee of the Third Military Medical University. All of the human brain tissue was obtained and used in a manner compliant with the Declaration of Helsinki. No tissue was resected solely for experimental purposes. A total of 16 TSC surgical specimens were obtained from patients undergoing surgery for intractable epilepsy. All of the cases were independently reviewed by two neuropathologists, and the diagnoses of TSC were in accordance with the diagnostic criteria for TSC (Crino et al., 2006). Furthermore, clinical mutation analyses of the TSC1 and TSC2 loci were performed by means of denaturing high performance liquid chromatography (DHPLC) to confirm our diagnoses. In this study, all of the patients did not have seizure activity in the last 24 h before surgery. Seizure outcome was assessed using Engel's et al. criteria. All patients underwent surgery and had a follow-up at least 1 year later. The detailed clinical data for each specimen are listed in Table 1.

For comparison, normal-appearing cortex (i.e., CTX) and white matter were obtained at autopsy from 10 patients (5 female, 5 male; mean age: 5.5 years, range: 2.4–10.8 years) who did not have a history of seizures or other neurological diseases. All of the autopsies were performed within 6 h of death. Two neuropathologists also helped to review the control cases, and both gross and microscopic examinations revealed no abnormalities. The clinical data for the normal control tissues are summarized in Supplemental Table 1.

2.2. Tissue preparation

All brain samples obtained at surgery or autopsy were immediately divided into two parts. One part was immediately placed in a cryovial that had been soaked in buffered diethylpyrocarbonate (1:1000) for 24 h and was then snap-frozen in liquid N_2. The frozen samples were maintained at −80 °C until they were used for Western blotting. The remaining part of the sample was fixed in 10% buffered formalin for 24 h and was then embedded in paraffin. The paraffin-embedded tissue was sectioned at 5 μm for histological and immunohistochemical staining, or 10 μm for double immunofluorescence staining.

2.3. Western blotting analysis

Western blotting analyses were performed to quantify the amount of IL-17 and IL-17R protein in homogenates from TSC cortical tubers ($n = 16$), and CTX samples ($n = 10$). β-actin levels were evaluated as a loading control. The frozen samples were dissected on a freezing table and homogenized. The tissue homogenates were lysed in RIPA buffer containing 10 mM Tris (pH 8.0), 150 mM NaCl, 10% glycerol, 1% NP-40, 0.4 mg/ml Na-orthovanadate, 5 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 5 mM NaF and 10% protease inhibitor cocktail (Sigma, St. Louis, MO). The protein concentration was determined using the bicinchoninic acid protein assay (Bio-Rad, Hercules, CA, USA). For electrophoresis, equal amounts of protein (30 μg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis in 6%, 10% or 12% polyacrylamide gel depending on the target protein. The separated proteins were transferred onto polyvinylidene fluoride membranes (Millipore, Temecula, CA, USA) using a semi-dry electroblotting system (Transblot SD; Bio-Rad). For immunoblotting, the membranes were blocked in 5% nonfat dry milk for 1 h and incubated overnight at 4 °C with one of the following primary antibodies: β-actin (rabbit monoclonal, 1:1000; Santa Cruz, CA, USA), IL-17 (rabbit monoclonal, 1:1000; Millipore), or IL-17R (rabbit polyclonal, 1:400; Santa Cruz). After several washes in Tris-buffered saline containing 0.5% Tween-20 (TBST), the samples were treated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:1000; Zhongshan Goldenbridge Biotechnology Co, Beijing, China) for 1 h at room temperature. The antibody labeling was visualized using enhanced chemiluminescence.

Table 1
Clinical and neuropathological characteristics of patients with TSC.

Case no.	Sex	Pathology	Genotype	Age at surgery (year)	Epilepsy duration (years)	Seizure type	Epileptogenic lesion location	Seizure frequency (per month)	AEDs	PO	Application in present study
1	F	TSC	TSC2	1.1	0.8	IS	F	75	VPA, CBZ, CLZ, ACTH	II	WB, IHC
2	M	TSC	TSC1	1.8	0.9	PS	P	25	VPA, CLZ	I	WB, IHC
3	M	TSC	TSC2	2.5	0.8	PS	P	10	VPA, TPM, LMT	I	WB, IHC
4	F	TSC	TSC1	3.1	2.5	IS, GTCS	F	10	VPA, ACTH, PHT, LMT	II	WB, IHC
5	M	TSC	TSC2	4.2	2.5	PS, IS	T	135	TPM, LEV, ACTH	I	WB, IHC
6	F	TSC	TSC1	4.6	3.5	PS, GTCS	O	30	CBZ, VPA, ACTH	I	WB, IHC
7	F	TSC	TSC2	4.6	3.5	PS, IS	T	120	VPA, TPM, LMT	III	WB, IHC
8	M	TSC	TSC2	5.2	3.5	PS	F, O	30	VPA, LEV	I	WB, IHC
9	M	TSC	TSC2	5.6	4.5	GTCS	T	15	VPA, CLZ	I	WB, IHC
10	M	TSC	TSC2	6.4	4.3	PS	F	60	VPA, TPM, LMT	I	WB, IHC
11	F	TSC	TSC2	7.6	6.0	PS, GTCS	F	10	TPM, CBZ	I	WB, IHC
12	M	TSC	TSC2	8.2	6.9	PS	T	75	VPA, TPM, PB	I	WB, IHC
13	F	TSC	NMI	8.5	6.2	PS, Tonic	O	85	VPA, CLZ	II	WB, IHC
14	F	TSC	TSC1	9.7	3.0	PS	P, T	20	CBZ, VPA	I	WB, IHC
15	M	TSC	TSC2	11.3	4.9	Tonic	F	5	OxCZ, VPA	IV	WB, IHC
16	M	TSC	NMI	11.5	8	PS, GTCS, Tonic	T	10	VPA, LEV, CLZ	III	WB, IHC

TSC, tuberous sclerosis complex; AEDs, antiepileptic drugs; PO, postoperative outcome (Engel's class); F, female; M, male; NMI: No Mutation Identified by genetic analysis; GTCS, generalized tonic–clonic seizure; PS, partial seizure; IS, infantile spasm; F, frontal lobe; P, parietal lobe; O, occipital lobe; T, temporal lobe; PHT, phenytoin; CBZ, carbamazepine; PB, phenobarbital; TPM, topiramate; ACTH, adrenocorticotropic hormone; VPA, valproate; OxCZ, oxcarbazepine; LEV, levetiracetam; LMT, lamotrigine; CLZ, clonazepam; WB, Western blotting; IHC, immunohistochemistry (including immunofluorescence).