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

Jiao-Jiang He, Ke-Fu Wu, Song Li, Hai-Feng Shu, Chun-Qing Zhang, Shi-Yong Liu, Mei-Hua Yang, Qing Yin, Hui Yang

Department of Neurosurgery, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

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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 epileptogenesis of cortical tubers in TSC.

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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 (SEGs). 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...
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 epileptogenesis.

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 N2. 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 electroblootting 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     | 1.9                   | 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                      | GTCS         | O                            | 30                            | CBZ, VPA, ACTH               | I    | WB, IHC                     |
| 7        | F   | TSC       | TSC1     | 4.6                   | 3.5                      | IS           | T                            | 120                           | 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                      | 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       | TSC1     | 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                             | Oxoz, VPA                    | IV   | WB, IHC                     |
| 16       | M   | TSC       | TSC1     | 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; Oxoz, oxcarbazepine; LEV, levetiracetam; LMT, lamotrigine; CLZ, clonazepam; WB, Western blouting; IHC, immunohistochemistry (including immunofluorescence).
For the immunoblotting analyses, densitometry was performed with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The optical densities (ODs) of the protein bands were calculated relative to the ODs of the reference protein, β-actin.

2.4. Histology and immunohistochemistry

Paraffin sections were mounted on polylysine-coated slides. Two slices in each paraffin block were routinely stained with hematoxylin and eosin (H&E), and consecutive serial sections were used for immunohistochemistry (IHC). The paraffin-embedded sections were deparaffinized, rehydrated, and incubated for 30 min in 0.3% H2O2 diluted in methanol to quench any endogenous peroxidase activity. All of the samples were placed in phosphate buffered saline (PBS) (0.01 M, pH 7.3) and heated in a microwave oven for antigen retrieval. The sections were washed with PBS and incubated for 60 min in 10% normal goat serum at room temperature, followed by incubation with primary rabbit anti-human IL-17 (rabbit polyclonal, 1:100; Santa Cruz) or IL-17R (rabbit polyclonal, 1:100; Santa Cruz) overnight at 4 °C. After 3 rinses with PBS, the sections were incubated with goat anti-rabbit immunoglobulin conjugated to peroxidase-labeled dextran polymer (EnVision + System-HRP; Boster, Wuhan, China) for 60 min at 37 °C. The immunoreactions were visualized using 3,3-diaminobenzidine (Dako, Glostrup, Denmark), anti-CD4 (mouse monoclonal, 1:50; Dako), anti-CD8 (mouse monoclonal, 1:40; Dako), and anti-IL-17R combined with anti-GFAP or anti-NF200. After 3 washes with PBS, the sections were incubated for 1 h at room temperature followed by over-night incubation at 4 °C with primary anti-IL-17 combined with anti-GFAP (mouse monoclonal, 1:500; Sigma), anti-NF200 (mouse monoclonal, 1:100; Boster), anti-HLA-DR, DQ, DR (mouse monoclonal, 1:100, Dako, Glostrup, Denmark), anti-CD4 (mouse monoclonal, 1:50; Dako) or anti-CD8 (mouse monoclonal, 1:40; Dako) and anti-IL-17 combined with anti-GFAP or anti-NF200. After 3 washes with PBS, the sections were incubated with a mixture of FITC-conjugated goat anti-rabbit IgG (1:300; Zhongshan Goldenbridge Biotechnology Co) and Alexa Fluor 594 goat anti-mouse IgG (1:500, Invitrogen) for 1 h at 37 °C. Next, the sections were mounted with Vectashield containing DAPI (10 μg/ml, Beyotime, China) to label DNA in cell nuclei. The fluorescence sections were observed and photographed with a confocal laser scanning microscope (TCS-TIV; Leica, Nussloch, Germany).

2.5. Evaluation of immunostaining and cell counting

All of the labeled tissue sections were evaluated as previously reported (Zurolo et al., 2011) using a Leica DMI82B microscope to examine a total microscopic area of 781.250 μm² (200 high-power non-overlapping fields of 0.0625 x 0.0625 mm width, using a square grid inserted into the eyepiece). The intensity of the IL-17 and IL-17R immunoreactivity (IR) was evaluated using a semi-quantitative three-point scale in which IR was defined as follows: — absent (0), + weak (1), ++ moderate (2), or +++ strong (3) (Table 2). These scores represent the predominant staining intensity in each section and were calculated as the averages of the selected fields. Additionally, we calculated the labeling index (LI) of IL-17 and IL-17R positive cells in the TSC cortical tubers as previously described (Schick et al., 2007). The LI was defined as the ratio of the immunolabeled cells to the entire target cell population.

Table 2

|           | CTX (n = 10) | TSC (n = 16) |
|-----------|-------------|-------------|
| IL-17     | 0.83 ± 0.24 | 2.27 ± 0.42 |
| IL-17R    | 0.62 ± 0.17 | 2.36 ± 0.25 |

Data are expressed as mean ± SEM. **P < 0.001, TSC versus CTX, ANOVA.

3. Results

3.1. Case material and histological features

The clinical features of the tissues obtained from the TSC that were used in this study are summarized in Table 1. All of the patients underwent surgery for intractable epilepsy, and most were completely seizure-free following surgery. The cortical tubers of TSC cases in this study were histologically composed of abnormal cortical laminar architecture, astrogliosis, DNs, and GCs (see Supplemental Fig. 1B). The DNs were identified as neurons with abnormal morphology, enlarged somal size, abnormal orientation, abnormal dendritic patterns, and abundant Nissl substances. The GCs, a unique cell type, were defined as morphologically abnormal cells with a thin membrane, eosinophilic cytoplasm, no clear axonal or dendritic processes, and one or more eccentrically located nuclei. At low magnification, the CTX with H&E staining has organized layers with well-preserved laminations and a unipolar orientation of apical dendrites toward the pial surface as well as normal definition of the gray-white matter junction (see Supplemental Fig. 1A).

3.2. Western blotting analysis of IL-17 and IL-17R

We examined the expression of the IL-17 at the protein level by Western blot analysis in cortical tubers of TSC. Fig. 1A shows that IL-17 and IL-17R were present as bands of approximately 32 kDa and 120 kDa, respectively. There was a notable increase in IL-17 and IL-17R expression in the cortical tubers compared with CTX samples (Fig. 1B).

Fig. 1. Changes in IL-17 and IL-17R expression in cortical tubers of TSC. (A) Representative immunoblot bands and (B) densitometric analyses of total homogenates from TSC tubers and CTX tissues. *P < 0.05 and **P < 0.01 versus CTX, ANOVA.
3.3. IL-17 immunoreactivity in cortical tubers of TSC

Weak to moderate IL-17 IR was detected in neurons and glial cells throughout all of the cortical layers in the CTX specimens (Fig. 2A, B); weak staining was observed in the endothelial cells of blood vessels (Fig. 2A, inset).

In the cortical tuber, there was strong IL-17 IR in 89% ± 2.1% of the DNs (n = 584) (Fig. 2C, D) and in 91% ± 1.3% of the GCs (n = 409) (Fig. 2C, D), along with moderate to strong IL-17 IR in the glial cells (Fig. 2C, D). Additionally, strong IL-17 IR staining was found in the endothelial cells of blood vessels (Fig. 2C, inset). The intensity scores of IL-17 IR in the cortical tuber were dramatically higher than those in the CTX samples (Table 2). Double-labeling experiments revealed that NF200-positive (NF200+) DNs (Fig. 2F) and GCs (Fig. 2G, H) both expressed IL-17. Moreover, most of the IL-17+ glial cells were the GFAP+ astrocytes (Fig. 2E) and HLA+ microglia (Fig. 2I). In this study, we also observed a few CD4+ (Fig. 2J) but not CD8+ (Fig. 2K) cells that expressed IL-17 in the TSC specimens.

3.4. IL-17R immunoreactivity in cortical tubers of TSC

In the control specimens, weak to moderate IL-17R IR expression was detected in neurons throughout all of the cortical layers (Fig. 3A).

Fig. 2. Cell-specific distribution of IL-17 in cortical tubers of TSC. (A and B) IL-17 IR in CTX. Weak to moderate IL-17 IR in neurons (arrows in A) and glial cells (arrowheads in B), and weak IL-17 IR in endothelial cells (inset, A) in gray matter (GM) and white matter (WM). (C and D) IL-17 IR in TSC. Strong IL-17 IR in glial cells (arrowheads in C and D), DNs (arrows in C, D and inset b in D) and GCs (double arrows in C, D and inset a in D). Moderate to strong IL-17 IR in endothelial cells (inset, C, E and I). The merged images show the colocalization of IL-17 (green) with GFAP (red) in glial cells (arrowheads in E) and HLA (red) in microglia (arrowheads in I) but not in DNs (arrows in I). (F–H) Confocal image showing IL-17 IR DNs (F) and GCs with different sizes and shapes (G and H) colabeled with NF200 (red). (J and K) Double labeling staining shows the colocalization of IL-17 (green) with CD4 (red, arrows in J) but not CD8 (red, arrows in K) in T-lymphocytes. Sections are counterstained with hematoxylin (A–D) or DAPI (E–K). The bars indicate (A–D) 50 μm; (E, K) 30 μm; and (F–J) 20 μm.
Occasionally, weak staining could be observed in the glial cells (Fig. 3B) and in the endothelial cells of blood vessels (Fig. 3B, inset).

In the cortical tuber, there was a moderate to strong IL-17R IR in 85% ± 2.3% of the GCs (n = 427) (Fig. 3C), and strong expression was detected in 87% ± 1.5% of the DNs (n = 606) (Fig. 3D) and in the glial cells (Fig. 3C). In addition, moderate to strong staining was observed in the endothelial cells of blood vessels (Fig. 3C, inset). The intensity scores indicated an upregulation of IL-17R expression in the TSC samples compared with the CTX samples (Table 2).

Double-labeling experiments revealed that IL-17R and GFAP were co-expressed in astrocytes (Fig. 3E). Moreover, most IL-17R+ GCs (Fig. 3F) and DNs (Fig. 3G) were NF200+.

4. Discussion

Recent observations indicate that activation of both the innate and adaptive immune responses has been accepted as a striking feature that occurs in epilepsy-associated cortical tuber and proinflammatory cytokines may play critical roles in epileptogenesis (Boer et al., 2008; Vezzani et al., 2008, 2011). In the present study, we found that the expression of IL-17 and IL-17R is upregulated in cortical tubers of TSC at protein levels. Intriguingly, IL-17 and IL-17R are expressed at high levels in misshapen cells (such as DNs, GCs) and glial (such as reactive astrocytes and microglia) as well as the endothelial cells of blood vessels. The cell-specific distribution pattern of the IL-17 system in cortical tuber of TSC suggests that it may be involved in the epileptogenicity of cortical tuber in TSC.

Previous studies observed that IL-17 is upregulated in several neurological disorders such as autoimmune diseases, neurodegeneration, stroke and refractory epilepsy with FCDs (Wang et al., 2009; Xu and Cao, 2010; Muls et al., 2012; He et al., 2013). In our study, high levels of IL-17 protein were detected in cortical tuber tissue homogenate. Moreover, immunohistochemistry analyses showed that the IL-17 IR was generally located in misshapen cells (such as DNs and GCs) and glial. Intriguingly, there was coexpression of the neuronal markers NF200, but not the astrocytic marker GFAP, in IL-17+ DNs and GCs, suggesting that they have a neuronal lineage in TSC. Meanwhile, we found that the GFAP+ reactive astrocytes and the HLA+ microglia expressed high levels of IL-17 in cortical tubers of TSC. In the brain, innate immunity cell types comprise resident neurons, astrocytes, and microglia, which produce proinflammatory cytokines (Granata et al., 2011). All these suggested that innate immunity cells are the resources of IL-17 and it may participate in innate immunity response in TSC.

Nevertheless, the mechanisms of IL-17 upregulation in TSC are not clear. Abundant evidence indicates that cortical tubers are the epileptogenic foci in TSC (Wang et al., 2007). Seizure activity and abnormal

Fig. 3. Cell-specific distribution of IL-17R in cortical tubers of TSC. (A and B) IL-17R IR in CTX. Weak to moderate IL-17R IR in neurons (arrows in A) and weak IL-17R IR in glial cells (arrowheads in A and B), in endothelial cells (inset, B) in gray matter (GM) and white matter (WM). (C and D) IL-17R IR in TSC. Strong IL-17R IR in glial cells (arrowheads in C), GCs (double arrows in C) and DNs (arrows in D) with different shapes and sizes. Moderate to strong IL-17R IR in endothelial cells (inset, C). (E) The merged images show the colocalization of IL-17R (green) with GFAP (red) in glial cells (arrowheads) but not in GCs (arrows). (F and G) Confocal images showing the colocalization of IL-17R (green) with NF200 (red) in GCs (F) and in DNs (G). Sections are counterstained with hematoxylin (A–D) or DAPI (E–G). The bars indicate (A–D) 50 μm; (E) 30 μm; and (F, G) 20 μm.
glioneuronal proliferation are respectively the common clinical symptom and histological features of epilepsy patients with TSC and all of the samples were obtained from lesionectomy in this study. It is therefore possible that misshapen cells, astrocytes, and microglia in cortical tubers may also express and release IL-17 in response to seizure activities.

Recent studies indicated that the mTOR pathway not only plays a role in regulating cell growth and size (Fingar et al., 2002), but also regulates the induction of inflammatory mediators (Potter et al., 2001; Lim et al., 2003). Yurchenko et al. found that rapamycin strongly inhibits the induction of IL-17 (Yurchenko et al., 2012). Thus, the activation of mTOR pathway due to the mutation of TSC1 and/or TSC2 genes may contribute to the up-regulation of IL-17 in TSC.

We found that the IL-17 IR was generally located in misshapen cells (such as DNs and GCs) and glial. Misshapen cells may be the intrinsic “pacemakers” that initiate and drive the epileptiform activity in TSC (Cepeda et al., 2003; Wong, 2008). The activation of astrocytes and microglia is represented within cortical tubers and is involved in the generation of seizures (Wong; Najar et al., 2011). Our previous study and other groups have provided evidence indicating that IL-6 and IL-1β are overexpressed in cortical tubers of TSC and may contribute to the high epileptogenicity of the cortical tubers in TSC through their functional receptors (Ravizza et al., 2006; Shu et al., 2010), both of which could be induced by IL-17 (Jovanovic et al., 1998; Ma et al., 2010). Moreover, Richter et al. reported that IL-17 increased the excitability of dorsal root ganglia (DRG) neurons through IL-17R (Richter et al., 2012). It is likely, therefore, that the persistent upregulation of IL-17 and IL-17R may be involved in the epileptogenicity of cortical tubers in TSC.

Autocrine or paracrine activation of IL-17R in response to IL-17 released by neurons and/or glial cells may contribute to the recurrence or exacerbation of seizure activity in TSC.

In this study, the upregulated expressions of IL-17 and IL-17R were observed in the endothelial cells of blood vessels in TSC. Kebrí et al. have demonstrated that IL-17 promotes BBB disruption via binding with IL-17R in human endothelial cells (Kebrí et al., 2007). Recent studies using IL-17 knock-out mice demonstrated the prevention of experimental autoimmune encephalomyelitis induced BBB disruption (Huppert et al., 2010). Boer et al. reported BBB disruption in cortical tubers of TSC, as demonstrated by perivascular parenchymal leakage of serum albumin, with uptake into astrocytes (Boer et al., 2008). Interestingly, albumin uptake into astrocytes has been shown to affect K+ homeostasis, facilitating neuronal hyperexcitability and epileptiform activity (Ivens et al., 2007). Therefore, we speculate that IL-17 disrupts the BBB through interaction with IL-17R in endothelial cells and, subsequently, exacerbates seizure activity in TSC.

CD4+ and CD8+ T-lymphocytes were detected within TSC and FCDII cortical lesions (Boer et al., 2008; Iyer et al., 2010). Interestingly, we found that CD4+ but not CD8+ T-lymphocytes expressed high levels of IL-17 in the TSC specimens, suggesting that adaptive immune cells are the resources of IL-17 and it may participate in adaptive immunity response and adaptive immunity response as well as BBB disruption. Accordingly, we speculate that IL-17 may be involved in the epileptogenicity of cortical tubers in TSC by accelerating the severity of inflammatory response and BBB disruption, which requires further investigation in animal models of epilepsy.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jneuroim.2013.05.007.

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