The Interleukin 17 System in Cortical Lesions in Focal Cortical Dysplasias

Jiao-Jiang He, PhD, Song Li, MD, Hai-Feng Shu, MD, Si-Xun Yu, PhD, Shi-Yong Liu, MD, Qing Yin, MD, and Hui Yang, MD, PhD

Abstract
Focal cortical dysplasias (FCDs) are increasingly recognized as important causes of medically intractable epilepsy. To understand the potential role of the interleukin 17 (IL-17) system in the epileptogenesis of FCDs, we studied the expression patterns of the IL-17 system in 15 FCD type Ia (FCDIa), 12 FCD type Ila (FCDIla), and 12 FCD type IIb (FCDIIb) cortical lesions and compared the results with those in cerebral cortex from 10 control patients. Protein levels of IL-17, IL-17 receptor (IL-17R), and downstream factors of the IL-17 system in 15 FCDIa, FCDIla, and FCDIIb. Moreover, protein levels of IL-17 and IL-17R positively correlated with the frequency of seizures in FCD patients. Immunostaining indicated that IL-17 and IL-17R are highly expressed in neuronal microcolumns, dysmorphic neurons, balloon cells, astrocytes, and vascular endothelial cells. Nuclear factor-

INTRODUCTION
Focal cortical dysplasias (FCDs) are increasingly recognized as an important cause of epilepsy. The clinical significance and impact of FCDs are high because they are strongly associated with pharmacoresistant epilepsy. A subset of epilepsy patients with FCDs may be candidates for epilepsy surgery (1, 2). Recently, the International League Against Epilepsy Task Force proposed a new classification system based on neuropathologic examination of surgical specimens, electroclinical presentation, imaging, and postsurgical outcome. Focal cortical dysplasia type I (FCDI) refers to isolated lesions with abnormal cortical layering or compromised radial migration and maturation of neurons, the 6 layered tangential composition of the neocortex, or a combination of both variants. Focal cortical dysplasia type II (FCDII) is a malformation characterized by disrupted cortical lamination and specific cytopathologic abnormalities, which is differentiated into type Ia (FCDIa) with dysmorphic neurons (DNs) without balloon cells (BCs) and type IIb (FCDIIb) with DN and BCs (3). Several studies have indicated that patients with FCDI and FCDII have high frequencies of seizures (4, 5). An understanding of the molecular events that underlie the occurrence of seizures is essential for devising new therapeutic approaches for the treatment of epilepsy.

Increasing evidence indicates that proinflammatory cytokines are involved in the pathophysiology of epileptic disorders, including FCDs (6). Proinflammatory cytokines such as interleukin 1β (IL-1β), IL-6, and tumor necrosis factor (TNF) have long been thought to play an important role in epileptogenesis (7, 8). Interleukin 17, also referred to as IL-17A, is a prototypic member of the newest subclass of cytokines. Interleukin 17 acts as a proinflammatory cytokine that can induce the release of certain chemokines and cytokines (e.g. IL-1β, IL-6, and TNF) and has a distinct ligand-receptor system (9). Several studies have demonstrated that IL-17 can play a bridging role between innate and adaptive immunity in vivo and that IL-17 promotes neuronal injury through an IL-17/IL-17 receptor (IL-17R) combination in conditions such as multiple sclerosis and ischemic brain injury (9–11). An increasing number of observations suggest that activation of both the innate and adaptive immune responses occurs in FCDs and that the inflammatory response may contribute to the generation and recurrence of seizures (6, 12).

We hypothesized that IL-17 may play a role in the epileptogenesis of FCDs. However, to our knowledge, there are no reports examining the role of IL-17 system signaling in FCDs. Therefore, we analyzed surgically resected FCDs to determine the protein levels of IL-17, IL-17R, and the downstream factors of IL-17 system signaling and compared...
them with normal-appearing control cortex (CTX). In addition, we investigated the specific cellular distribution of IL-17, IL-17R, and its downstream factors in FCDIa, FCDIIa, and FCDIIb.

**MATERIALS AND METHODS**

**Subjects**

A total of 39 surgical specimens (15 FCDIa, 12 FCDIIa, and 12 FCDIIb) were obtained from patients undergoing surgery for intractable epilepsy. In addition to lesionectomy (n = 34), 5 patients had multilobar resections. 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 brain tissues were obtained and used in a manner compliant with the Declaration of Helsinki. All of the cases were independently reviewed by 2 neuropathologists, and the diagnoses were confirmed using the current International League Against Epilepsy classification system for grading FCDs. The clinical features of the FCD patients are summarized in Table 1. Detailed clinical data for each FCD patient specimen are listed in Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A424. The mean length of postoperative follow-up was 3.3 years (range, 1–7 years).

Control CTX and white matter were obtained at autopsy from 10 patients (6 female, 4 male; mean age, 5.7 years; range, 2.1–10.8 years), without a history of seizures or other neurologic diseases (Table 2). All of the autopsies were performed within 6 hours of death. Within this postmortem interval, the levels of IL-17 were stable. The levels of IL-17 in the CTX from patients who died of drowning and other circumstances are not significantly different (data not shown). Two neuropathologists also reviewed the control case specimens, and both gross and microscopic examinations revealed no abnormalities. Clinical data for the normal control tissue patient sources are summarized in Table 2.

**Tissue Preparation**

All of the FCD brain samples were immediately divided into 2 parts. One part was fixed in 10% buffered formalin for 24 hours and was then embedded in paraffin. The paraffin-embedded tissue was sectioned at 5 μm for subsequent histologic and immunohistochemical staining. The remaining part of the sample was immediately placed in a cryovial that had been soaked in buffered diethylpyrocarbonate (1:1000) for 24 hours and was then snap-frozen in liquid N2. The frozen samples were maintained at -80°C until they were used for Western blotting.

**Western Blotting**

Western blotting analyses were performed to quantify the amount of IL-17, IL-17R, nuclear factor-κB activator 1 (NFκB; ACT1) and NFκB-p65 protein in homogenates from FCDIa cortical lesions (n = 12), FCDIIa cortical lesions (n = 10), FCDIIb cortical lesions (n = 10), and CTX samples (n = 10). Levels of β-actin were evaluated as a loading control. The frozen samples were dissected on a freezing table and homogenized. The tissue homogenates were lysed in RIPA (radioimmunoprecipitation assay) buffer containing 10 mmol/L Tris (pH 8.0), 150 mmol/L NaCl, 10% glycerol, 1% NP-40, 0.4 mg/mL Na-orthovanadate, 5 mmol/L EDTA (pH 8.0), 5 mmol/L NaF, and 10% protease inhibitor cocktail (Sigma, St. Louis, MO). The total tissue lysates were centrifuged at 20,000 × g for 15 minutes at 4°C, after which the protein concentration in the supernatant was estimated using the bicinchoninic acid protein assay (Bio-Rad, Hercules, CA). For electrophoresis, equal amounts of protein (30 μg/lane) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis in 6%, 10%, or 12% polyacrylamide gel, depending on the target protein. The separated proteins

**TABLE 1. Summary of Focal Cortical Dysplasia Patient Clinical Features**

<table>
<thead>
<tr>
<th></th>
<th>FCDIa (n = 15)</th>
<th>FCDIIa (n = 12)</th>
<th>FCDIIb (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>9:6</td>
<td>6:6</td>
<td>7:5</td>
</tr>
<tr>
<td>Age at surgery, mean (range), years</td>
<td>16.1 (2.6–31.5)</td>
<td>6.4 (1.2–11.5)</td>
<td>5.2 (1.2–8.5)</td>
</tr>
<tr>
<td>Seizure type, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS (80)</td>
<td>47</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>GTCS (47)</td>
<td>27</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Tonic (27)</td>
<td>IS (27)</td>
<td>IS (25)</td>
<td>IS (25)</td>
</tr>
<tr>
<td>Cortical lesion location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal: 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporal: 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parietal: 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occipital: 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of epilepsy, mean (range), years</td>
<td>11.7 (1.9–23)</td>
<td>5.3 (1–9.5)</td>
<td>4.1 (1–8)</td>
</tr>
<tr>
<td>Seizure frequency, mean (range), per month</td>
<td>32 (3–115)</td>
<td>55 (8–205)</td>
<td>62 (10–175)</td>
</tr>
<tr>
<td>Postoperative outcome: Engel class, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I: 45</td>
<td>I: 67</td>
<td>I: 50</td>
<td></td>
</tr>
<tr>
<td>II: 15</td>
<td>II: 18</td>
<td>II: 33</td>
<td></td>
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<tr>
<td>III: 20</td>
<td>III: 12</td>
<td>III: 8</td>
<td></td>
</tr>
<tr>
<td>IV: 20</td>
<td>IV: 13</td>
<td>IV: 9</td>
<td></td>
</tr>
</tbody>
</table>

GTCS, generalized tonic-clonic seizure; IS, infantile spasm; PS, partial seizure.
were transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA) using a semidy electroblotting system (Transblot SD; Bio-Rad). For immunoblotting, the membranes were blocked in 5% dry milk for 1 hour and incubated overnight at 4°C with one of the primary antibodies to the following: β-actin (rabbit monoclonal, 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), IL-17 (rabbit polyclonal, 1:500; Santa Cruz Biotechnology), IL-17R (rabbit polyclonal, 1:400; Santa Cruz Biotechnology), ACT1 (rabbit polyclonal, 1:500; Sigma), or NFκB-p65 (rabbit monoclonal, 1:1000; Epitomics, Burlingame, CA). After several washes in Tris-buffered saline containing 0.5% Tween-20, the samples were incubated with horseradish peroxidase–conjugated goat anti-rabbit secondary antibody (1:1000; Zhongshan Goldenbridge Biotechnology Co., Beijing, China) for 1 hour at room temperature. The antibody labeling was visualized using enhanced chemiluminescence.

For the immunoblotting analyses, densitometry was performed with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The optical densities of the protein bands were calculated relative to the optical densities of the reference protein β-actin.

Histology and Immunohistochemistry
Paraffin sections were mounted on polylysine-coated slides. Two slices in each paraffin block were routinely stained with hematoxylin and eosin, and consecutive serial sections were used for immunohistochemistry. The paraffin-embedded sections were deparaffinized, rehydrated, and incubated for 30 minutes in 0.3% H2O2 diluted in methanol to quench endogenous peroxidase activity. All of the samples were placed in PBS (0.01 mol/L, pH 7.3) and heated in a microwave oven for antigen retrieval. The sections were placed in PBS (0.01 mol/L, pH 7.3) and heated in a microwave oven for antigen retrieval. The sections were incubated with a mixture of FITC (fluorescein isothiocyanate)-conjugated goat anti-rabbit immunoglobulin G (1:300; Zhongshan Goldenbridge Biotechnology Co.) and Alexa Fluor 594 goat anti-mouse immunoglobulin G (1:500; Boster). The sections were counterstained with hematoxylin, dehydrated, and coverslipped. No immunoreactive cells were detected in the negative control experiments that included using secondary antibody alone, preabsorption with a 10-fold excess of specific blocking antigen, or incubation with an isotype-matched rabbit polyclonal antibody (Figure, Supplemental Digital Content 2, http://links.lww.com/NEN/A425). A Leica DMIRB microscope (Leica, Nussloch, Germany) was used to capture the images of the sections.

For double-immunofluorescence staining, sections were incubated for 1 hour at room temperature, followed by overnight incubation at 4°C with primary anti–IL-17 combined with anti–glial fibrillary acidic protein ([GFAP] mouse monoclonal, 1:500; Sigma), anti-NF200 (mouse monoclonal, 1:100; Boster), or anti-CD4 (mouse monoclonal, 1:50; Dako, Glostrup, Denmark) and anti–IL-17R combined with anti-GFAP or anti-NF200. After 3 washes with PBS, the sections were incubated with a mixture of FITC (fluorescein isothiocyanate)-conjugated goat anti-rabbit immunoglobulin G (1:300; Zhongshan Goldenbridge Biotechnology Co.) and Alexa Fluor 594 goat anti-mouse immunoglobulin G (1:500; Invitrogen, Carlsbad, CA) for 1 hour at 37°C. Next, the sections were mounted with Vectashield containing DAPI ([4′,6-diamidino-2-phenylindole] 10 μg/mL, Beyotime, Canada) to label DNA in cell nuclei. The fluorescent sections were observed and photographed with a confocal laser scanning microscope (TCS-TIV, Leica).

Evaluation of Immunostaining and Cell Counting
All of the labeled tissue sections were evaluated using a Leica DMIRB microscope to examine a total microscopic area of 781.250 μm² (200 high-power nonoverlapping fields of 0.0625 × 0.0625 mm width using a square grid inserted into the eyepiece) (13). The intensity of the IL-17, IL-17R, ACT1, and NFκB-p65 immunoreactivity (IR) was evaluated using a semiquantitative 3-point scale in which IR was defined as follows: 0, absent; +, weak; ++, moderate; or +++, strong (Table 3). These scores represent the predominant staining

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**TABLE 2. Clinical and Neuropathologic Features of Autopsy Control Patient Samples**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Neuropathologic Diagnosis</th>
<th>Age, years</th>
<th>Cause of Death</th>
<th>PMI</th>
<th>Cortical Region Sampled</th>
<th>Application in the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>Normal</td>
<td>2.1</td>
<td>Non-neurologic disease</td>
<td>0.5</td>
<td>Temporal; parietal; occipital</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Normal</td>
<td>3.0</td>
<td>Choking/suffocation</td>
<td>5.0</td>
<td>Parietal</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Normal</td>
<td>3.5</td>
<td>Electric shock</td>
<td>4.5</td>
<td>Frontal; temporal</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Normal</td>
<td>3.5</td>
<td>Electric shock</td>
<td>1.2</td>
<td>Frontal</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Normal</td>
<td>4.0</td>
<td>Non-neurologic disease</td>
<td>1.0</td>
<td>Frontal; temporal</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Normal</td>
<td>6.5</td>
<td>Motor vehicle accident</td>
<td>2.0</td>
<td>Temporal; occipital</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Normal</td>
<td>7.1</td>
<td>Drowning</td>
<td>6.0</td>
<td>Frontal; parietal</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>Normal</td>
<td>7.5</td>
<td>Drowning</td>
<td>4.0</td>
<td>Frontal; parietal</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>Normal</td>
<td>8.5</td>
<td>Drowning</td>
<td>3.0</td>
<td>Temporal; parietal; occipital</td>
<td>WB, IHC</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>Normal</td>
<td>10.8</td>
<td>Motor vehicle accident</td>
<td>2.5</td>
<td>Frontal; temporal</td>
<td>WB, IHC</td>
</tr>
</tbody>
</table>

F, female; IHC, immunohistochemistry (including immunofluorescence); M, male; PMI, postmortem interval, that is, interval between death of a patient and removal of the brain before freezing or fixation; WB, Western blot.
intensity in each section and were calculated as the averages of the selected fields. In addition, we calculated the labeling index of IL-17Y, IL-17R, ACT1-, and NFp65-positive cells in the FCD tissue (14). The Labeling Index was defined as the ratio of the immunolabeled cells to the entire target cell population.

**Data Analysis and Statistics**

Data are expressed as mean ± SEM. Statistical analyses were performed with the Statistical Products and Service Solution package (SPSS for Windows, version 13.0; SPSS, Inc., Chicago, IL). The differences between the experimental groups were analyzed by one-way ANOVA (analysis of variance). Correlations between the IL-17 system protein levels and different clinical variables were assessed using Spearman rank correlation test. \( p \leq 0.05 \) was considered to be significant.

**RESULTS**

**Neuropathology**

The FCDIa cases showed abundant microcolumnar organization in gray matter and heterotopic neurons in white matter. The border between gray matter and white matter was usually blurred because of the increased numbers of heterotopic neurons. The FCDIIa cases displayed cortical delamination and DNs that were disoriented, had enlarged cell bodies and nuclei, and were distributed throughout the entire cortical thickness or located within the white matter. The FCDIIb cases showed all of the previously described histopathologic features of FCDIIa, in addition to BCs with eccentric nuclei, large cell bodies, and opalescent glassy eosinophilic cytoplasm, as observed by hematoxylin and eosin staining (Figure, Supplemental Digital Content 3, http://links.lww.com/NEN/A426).

**Western Blotting Analysis of IL-17 and IL-17R**

In Western blot analysis of tissue homogenates, IL-17 and IL-17R were present as bands of approximately 17 kd and 120 kd, respectively (Fig. 1A). The IL-17 and IL-17R protein levels in the FCDIa, FCDIIa, and FCDIIb cortical lesions were significantly higher than in the CTX samples (Fig. 1B). Importantly, the expression levels of IL-17R were prominently increased in the FCDIIa and FCDIIb samples versus the FCDIa samples (Fig. 1B).

**IL-17 Immunoreactivity**

In the control specimens, there was weak to moderate IL-17 IR detected in neurons and glial cells throughout all of the cortical layers (Fig. 2A; C); there was weak staining in vascular endothelial cells (Fig. 2B). In the FCDIa cortical lesions, there was moderate to strong staining for IL-17 in the neurons, including in the microcolumns, which are a distinctive feature of FCDIa (Fig. 2D). Concurrently, strong staining was detected in the glial cells (Fig. 2E). In addition, there was moderate to strong staining in vascular endothelial cells.
IL-17R Immunoreactivity

Weak to moderate IL-17R IR expression was detected in neurons throughout all of the cortical layers in the CTX specimens (Fig. 3A). Occasionally, there was weak staining in glia and vascular endothelial cells (Fig. 3A, B).

In FCDIa lesions, there was strong IL-17R staining in the neurons, including in the microcolumns in the gray matter (Fig. 3C) and in heterotopic neurons in the white matter (Fig. 3D). Moderate IL-17R staining was detected in the glial cells (Fig. 3D), and moderate to strong staining was observed in vascular endothelial cells (Fig. 3E). The intensity scores indicated a higher expression of IL-17R in the FCDIa samples versus the CTX samples (Table 3). Double labeling experiments confirmed the colocalization of IL-17R IR with the neuronal marker NF-200 in neurons (Fig. 3F–H) and with the astrocytic marker GFAP in astrocytes (Fig. 3I).

In FCDIb, there was strong IL-17R expression in 84% ± 7.3% of the DNs (n = 842) (Fig. 3J–L) and in glial cells (Fig. 3I, inset). As in the FCDIa specimens, moderate to strong IL-17R staining was found in vascular endothelial cells (Fig. 3M). The intensity scores indicated upregulation of IL-17R expression in the FCDIb samples compared with the CTX samples (Table 3). Double labeling experiments demonstrated that most NF200-positive DNs were IL-17R positive (Fig. 3N). Moreover, IL-17R and GFAP were coexpressed in astrocytes in the FCDIb specimens (Fig. 3O).

FIGURE 2. Immunoreactivity (IR) of interleukin 17 (IL-17) in focal cortical dysplasia (FCD) types Ia (FCDIa), Ib (FCDIb), IIa (FCDIIa), and IIb (FCDIIb). (A–C) Interleukin 17 (IL-17) in normal control cortex (CTX). There is weak to moderate IL-17 IR in neurons (arrows in [A] and [C]) and glial cells (arrowheads in [B] and [C]) and weak IL-17 IR in endothelial cells (double arrows in [B]) in neocortex, white matter (WM), and junction. (D, E) Interleukin 17 IR in FCDIa. Moderate to strong IL-17 IR in neurons, including in microcolumns (D) and in endothelial cells ([E] inset), and strong IL-17 IR in glial cells (arrowheads in [E]). (F, G) Merged images show the colocalization of IL-17 (green with NF200 (red) in neurons (F) and the colocalization of IL-17 (green) with glial fibrillary acidic protein ([GFAP] red) in glial cells (arrowheads) but not in neurons (arrows in [G]). (H, I) Interleukin 17 IR in FCDIIa. Moderate to strong IL-17 IR in dysmorphic neurons (DNs) (arrows in [H] and [I]) and in endothelial cells ([H] inset b); strong IL-17 IR in glial cells (arrowheads in [H]). The merged images show the colocalization of IL-17 (green) with NF200 (red) in DNs ([I] inset). (J) Confocal images showing that IL-17 (green) colocalizes with GFAP (red) in glial cells (arrowheads) but not in DNs (arrows) and colocalization of IL-17 (green) with CD4 (red) in T lymphocytes ([J] inset b). (K, R) Interleukin 17 IR in FCDIIb. Moderate to strong IL-17 IR in endothelial cells ([M], DNs (arrows in [K], [P], and [Q]), and balloon cells (BCs) double arrowheads in [K], [L], [N], and [O]) with different shapes and sizes. Interleukin 17–negative BCs are indicated by triple arrowheads (O). There is strong IL-17 IR in glial cells (arrowheads in [L] and [R]). (S) Confocal image showing that IL-17–positive DNs (arrows) and certain BCs (double arrowheads) colocalize with NF200 (red); certain other IL-17 IR BCs (triple arrowheads) do not colocalize with NF200 (red). (T) Merged image shows that IL-17–positive glial cells (arrowheads, green) and certain IL-17–positive BCs (double arrowheads, green) colocalize with GFAP (red); other BCs (triple arrowheads, green) do not colocalize with GFAP; cells with IL-17 IR (green, T lymphocytes) colocalize with CD4 (red) ([T] inset). Sections are counterstained with hematoxylin (A, E, H, I, K, R) or DAPI (F, G, insets in I, J, S, T) Scale bars = (A–E, H, K, L, S, T) 50 μm; (G, I, J, M–R) 30 μm; (F) 20 μm.
Weak ACT1 IR was detected in neurons, glial cells, and endothelial cells in the control specimens (Fig. 5A). In the FCDIa cortical lesions, there was moderate to strong staining of ACT1 in the neurons, including in the microcolumns and glial cells (Fig. 5B). Strong staining was also detected in vascular endothelial cells (Fig. 5B, inset). In FCDIa, strong ACT1 IR was detected in 91% ± 2.3% of the DNs (n = 732), glial cells (Fig. 5C), and endothelial cells (Fig. 5C, inset). In FCDIIb, there was strong ACT1 IR in 95% ± 1.1% of the DNs (n = 719) and in 82% ± 4.2% of the BCS (n = 394) (Fig. 5D). Expression patterns of ACT1 in glial cells (Fig. 5D) and endothelial cells (Fig. 5D, inset) in FCDIIb were similar to those in FCDIa. The intensity scores of ACT1 IR in the cortical lesions of FCDIa, FCDIIa, and FCDIIb were significantly higher than those in the CTX samples (Table 3).

There was weak NFκB-p65 IR in neurons, glial cells (Fig. 5E), and endothelial cells (Fig. 5E, inset) in control specimens. In the FCDIa cortical lesions, there was moderate to strong cytoplasmic and nuclear staining for NFκB-p65 in neurons, including in microcolumns (Fig. 5F, inset). In addition, there was strong cytoplasmic and nuclear staining in endothelial cells (Fig. 5F, inset b) and glial cells (Fig. 5F, inset a). Moderate to strong NFκB-p65 expression was detected in 77% ± 5.9% (FCDIa, n = 796) and 72% ± 7.7% (FCDIIb, n = 743) of the DNs with both cytoplasmic and nuclear staining (Fig. 5G, H). Strong staining was also detected in 56% ± 6.5% of the BCS (n = 439) (Fig. 5H). There was also strong staining in endothelial cells (Fig. 5G, inset, H, inset a) and cytoplasm and nuclei of glial cells (Fig. 5G; H, inset a) in FCDIa and FCDIIb samples. The intensity scores indicated increased expression of NFκB-p65 in the FCDIa, FCDIIa, and FCDIIb samples compared to the CTX samples (Table 3).

**Correlation Between the Protein Levels of the IL-17 System and the Clinical Features of FCDs**

Correlations between IL-17 system protein levels and different clinical variables (seizure frequency, duration of epilepsy, age at surgery, postoperative outcome) of all the surgical specimens of FCDs were assessed. The protein levels of IL-17 and IL-17R in FCDs were positively correlated with

**FIGURE 3.** Immunoreactivity (IR) of interleukin 17 receptor (IL-17R) in focal cortical dysplasia types Ia (FCDIa), IIA (FCDIIa), and IIB (FCDIIb). (A, B) Interleukin 17R IR in normal control cortex (CTX). Weak to moderate IL-17R IR in neurons (arrows in [A]) and weak IL-17R IR in glial cells (arrowheads in [B]) and in endothelial cells (double arrows in [A]) in neocortex and white matter (WM). (C–E) Interleukin 17R IR in FCDIa. There is strong IL-17R IR in neurons, including microcolumns composed of neurons in the cortex (C) and heterotopic neurons in the white matter (arrows in [D]), moderate IL-17R IR in glial cells (arrowheads in [D]), and moderate to strong IL-17R IR in endothelial cells (E). (F–H) The merged images show the colocalization of IL-17R (green) with NF200 (red) in neurons (arrows) and (I) the colocalization of IL-17R (green) with glial fibrillary acidic protein (GFAP) (red) in glial cells (arrowheads). (J–M) Interleukin 17R IR in FCDIIa. Strong IL-17R IR in glial cells (arrowheads in [J]) and in dysmorphic neurons (DNs) (arrows in [J–L]) of different shapes and sizes; moderate to strong IL-17R IR in endothelial cells (M). (N, O) The merged images show the colocalization of IL-17R (green) with NF200 (red) in DNs (arrows in [N]) and colocalization of IL-17R (green) with GFAP (red) in glial cells (arrowheads in [O]). (P–S) Interleukin 17R IR in FCDIIb. Strong IL-17R IR in glial cells (arrowheads in [Q]) and in DNs (arrows in [P] and [S]) of different shapes and sizes; moderate to strong IL-17R IR in endothelial cells (I) inset) and in balloon cells (BCs) (double arrowheads in [P–R]). The merged images show the colocalization of IL-17R (green) with NF200 (red) in BCS (R) inset) and in DNs (S) inset). (T) Confocal images showing that IL-17R–positive glial cells (arrowheads, green) colocalize with GFAP (red), and that IL-17R–positive DNs (arrows, green) do not colocalize with GFAP (red). Sections are counterstained with hematoxylin (A–E, J–M, P–S) or DAPI (insets in [R], [S], [F–I], [N], [O], and [T]). Scale bars = (A–D, I, J, N–P, T) 50 μm; (E–H, K–M, J, Q–S) 30 μm.

**FIGURE 4.** Changes in nuclear factor-κB (NFκB) activator 1 (ACT1) and NFκB-p65 (p65) expression in focal cortical dysplasia types Ia (FCDIa), Ila (FCDIIa), and Iib (FCDIIb). (A) Representative immunoblot bands and (B) densitometric analyses of total homogenates from FCDIa, FCDIIa, and FCDIIb lesions and normal control cortex (CTX) tissue samples. **p < 0.01 versus CTX; # p < 0.05 versus FCDIa, ANOVA.

**FIGURE 5.** Representative images showing interleukin 17 receptor (IL-17R) immunoreactivity (IR) in neocortex and white matter (WM). (A) Interleukin 17R IR in normal control cortex (CTX). Weak IL-17R IR in neurons, including in microcolumns and in dysmorphic neurons (arrows, green) do not colocalize with glial fibrillary acidic protein (GFAP, red). Sections are counterstained with hematoxylin or DAPI (insets in [R], [S], [F–I], [N], [O], and [T]). Scale bars = (A–D, I, J, N–P, T) 50 μm; (E–H, K–M, J, Q–S) 30 μm.
the frequency of seizures before surgical resection (Fig. 6A, B; Spearman rank correlation coefficient: IL-17: FCDs, $r = 0.672$, $p < 0.01$; FCDIa, $r = 0.728$, $p < 0.01$; FCDIIa, $r = 0.616$, $p < 0.01$; FCDIIb, $r = 0.743$, $p < 0.01$; IL-17R: FCDs, $r = 0.583$, $p < 0.01$; FCDIa, $r = 0.879$, $p < 0.01$; FCDIIa, $r = 0.591$, $p < 0.01$; FCDIIb, $r = 0.673$, $p < 0.01$). There were no significant correlations between the protein levels of IL-17 and IL-17R in FCDs and other clinical variables such as duration of epilepsy, age at surgery, or postoperative outcome (data not shown).

**DISCUSSION**

We show that the expressions of IL-17 and IL-17R are upregulated in FCDIa, FCDIIa, and FCDIIb tissue compared with that in CTX. Intriguingly, the protein levels of IL-17 and IL-17R in FCDs positively correlated with the frequency of seizures before surgical resection (Fig. 6A, B; Spearman rank correlation coefficient: IL-17: FCDs, $r = 0.672$, $p < 0.01$; FCDIa, $r = 0.728$, $p < 0.01$; FCDIIa, $r = 0.616$, $p < 0.01$; FCDIIb, $r = 0.743$, $p < 0.01$; IL-17R: FCDs, $r = 0.583$, $p < 0.01$; FCDIa, $r = 0.879$, $p < 0.01$; FCDIIa, $r = 0.591$, $p < 0.01$; FCDIIb, $r = 0.673$, $p < 0.01$). There were no significant correlations between the protein levels of IL-17 and IL-17R in FCDs and other clinical variables such as duration of epilepsy, age at surgery, or postoperative outcome (data not shown).
seizures. Interleukin 17 and IL-17R were highly expressed in neuronal microcolumns, DNs, BCs, astrocytes, and vascular endothelial cells. In addition, the protein levels of ACT1 and NFkB-p65, important downstream factors of the IL-17 signaling pathway, were significantly increased in FCDs; ACT1 and NFkB-p65 were diffusely expressed in FCD cortical lesions.

The Role of IL-17 in Seizures

Innate immunity and adaptive immunity occur in FCDs; the inflammatory response may contribute to the generation and recurrence of seizures by increasing the permeability of the blood–brain barrier (BBB) and neuron circuit excitability and decreasing the seizure threshold (6). Moreover, anti-inflammatory treatments may reduce seizures in experimental models and in some cases of epilepsy (15). Interleukin 17 can play a bridging role between innate and adaptive immunity in vivo by inducing the release of certain chemokines and cytokines (9). For example, IL-17 stimulation of astrocytes causes an increase in the expression of chemokines and leads to the infiltration of peripheral leukocytes in CNS disorders (16). We speculate that high levels of IL-17 may be involved in promoting seizure activity in FCD lesions by inducing proinflammatory cytokines, including IL-1β, IL-6, and TNF, which can promote seizure activity (17-23). Indeed, IL-17 may be upregulated and play important roles in other neurologic diseases in which seizures occur (11, 24-27). In particular, a causal relationship has been suggested between the extent of cortical inflammation (thought to be mediated mainly by IL-17) and the occurrence of epilepsy in multiple sclerosis patients who developed seizures (24-26). Moreover, IL-17 is involved not only in inflammation in ischemic brain injury but also promotes neuronal injury through the IL-17/IL-17R combination (11, 28).

**FIGURE 6.** Correlation between the protein levels of interleukin 17 (IL-17) and IL-17 receptor (IL-17R) and seizure frequency in focal cortical dysplasia types la (FCDIa), Ila (FCDIla), and IIb (FCDIib). (A) Scatter plot showing the significant positive correlation between the protein levels (relative optical density [OD]) of IL-17 and seizure frequency (seizures per month) in FCDs. Spearman rank correlation coefficient: IL-17: FCDs, r = 0.672, p < 0.01; FCDIa, r = 0.728, p < 0.01; FCDIla, r = 0.616, p < 0.01; FCDIib, r = 0.743, p < 0.01. (B) Scatter plot showing the significant positive correlation between the protein levels of IL-17R and seizure frequency in FCDs. Spearman rank correlation coefficient: IL-17R: FCDs, r = 0.583, p < 0.01; FCDIa, r = 0.879, p < 0.01; FCDIla, r = 0.591, p < 0.01; FCDIib, r = 0.673, p < 0.01.

**IL-17 and IL-17R in Neuronal Cells of FCDs**

In FCDIa, IL-17 and IL-17R IR were located in microcolumns in the neocortex and in heterotopic neurons in the white matter; they were in DNs in FCDIla and FCDIib and BCs in the latter. Because DNs exhibit atypical hyperexcitable intrinsic membrane properties (29, 30), it is likely that the persistent neuronal upregulation of IL-17 and its signaling receptor, IL-17R, in human FCD tissue is intrinsic to the developmental lesion per se or induced by seizures, or both.

Interleukin 17 could regulate neurotrophic effects via the production of neurotrophic factors (18). In addition, different types of neurotrophic receptors are highly expressed within the neuronal components of FCDs (31, 32). Moreover, Wang et al (33) found that IL-17 could promote antiapoptotic gene expression through STAT3 activation. Accordingly, we speculate that IL-17 may influence the survival of the abnormal hyperexcitable neurons in FCDs, which may act as a persistent source of proinflammatory cytokines in the cortical lesion, thus contributing to an increase in excitability.

**IL-17 and IL-17R in Glial Cells in FCDs**

Recent findings indicate that glial cells represent the main source of brain IL-17 production under pathologic conditions, including multiple sclerosis and ischemic brain injury (24, 28). Many activated astrocytes are present within FCDs and are involved in the generation of seizures (22, 34). We found that, as in neurons, astrocytes in FCDs express high levels of IL-17 and IL-17R, suggesting that IL-17 may be upregulated in FCDs via autocrine or paracrine modes of action.

Interleukin 17 can activate inducible nitric oxide synthase in astrocytes, leading to the production of nitric oxide (35), which increases glutamate release (36, 37). In particular, IL-17 has been shown to enhance the IL-6/soluble IL-6...
receptor signaling cascade and the IL-6 positive-feedback loop in astrocytes (38). In addition, IL-17 activates glial cells and induces their expression of IL-6, IL-1β, and TNF (39). This interaction may amplify the inflammatory response and subsequently exacerbates seizure activity and the extent of lesions in FCD.

IL-17 and IL-17R in BCs

Balloon cells expressed high levels of IL-17 and IL-17R, suggesting that they represent an additional source of persistent IL-17 production in FCDIIb. Consistent with a previous study (40), we demonstrated that IL-17–positive BCs were colabeled with both the neuronal marker NF200 and glial marker GFAP, suggesting that they could be both neuronal and glial lineages. Single-cell patch-clamp studies of BCs have shown that these cells are electrically silent and devoid of synaptic inputs (41). The role of BCs in the epileptogenicity of lesions in FCDIIb is complex. Previous studies indicated that apoptotic markers (e.g. bel-XL, bax, bel-2, and p53) (42) and IL-1β and IL-6 (22, 23) are aberrantly expressed in BCs. Accordingly, we speculate that IL-17 may exacerbate these IL-17R–mediated processes in BCs, but the roles of IL-17 in BCs require further investigation.

IL-17 and IL-17R in Endothelial Cells

Several studies have indicated that proinflammatory cytokines caused by seizure activity can lead to BBB disruption (20). Changes in the permeability of the BBB to serum albumin may result in chronic neuronal hyperexcitability (43). Because they express IL-17R, endothelial cells seem to be a target of IL-17. Indeed, Kebir et al (44) demonstrated that IL-17 promotes BBB disruption via binding with IL-17R in human endothelial cells. Therefore, we speculate that IL-17 disrupts the BBB through interaction with IL-17R in endothelial cells and subsequently exacerbates seizure activity in FCDs. As described previously (12), CD4-positive T lymphocytes were detected within FCDII tissue (both FCDIIa and FCDIIb) but not in FCDIa tissue. Iyer et al (12) also found moderate to strong monocyte chemoattractant protein 1 (MCP-1) IR in FCDII specimens, but weak MCP-1 IR was observed in both control and FCDI specimens. Moreover, IL-17 promotes the transmigration of human ex vivo CD4-positive T lymphocytes via enhanced secretion of the chemokine MCP-1 (44). Together, these results suggest that IL-17 may promote the transmigration of CD4-positive T lymphocytes via the enhanced secretion of MCP-1 in FCD patients.

Activation of ACT1 and NFκB-p65 in FCDs

The formation of the IL-17/IL-17R complex results in the activation of the ACT1/NFκB pathway and induction of the expression of inflammation-related genes (45). We found a pronounced increase in ACT1 and NFκB-p65 protein levels in FCDs versus CTX tissues. Moreover, ACT1 and NFκB-p65 were widely distributed in the FCD cortical lesions. Our data indicate that, in FCDs, the activation of ACT1 and NFκB-p65 may be involved in the IL-17 signaling pathways, but the direct contribution of IL-17 to the activation of the ACT1/NFκB pathway in FCDs needs to be further investigated.

Correlation With Seizure Frequencies

Protein levels of IL-17 in FCDs were positively correlated with the frequency of seizures but not with the duration of epilepsy. We speculate that seizures are associated with a sudden and probably short-lived burst of IL-17 or that the upregulation of IL-17 in human FCD tissue was induced by the seizures. However, because of the observational nature of the present study, we cannot attribute causality to this association but can only report this correlation. Interpretation of the significance of these findings may be elucidated in studies of animal epilepsy models.

In conclusion, we report that the IL-17 system is overexpressed in FCDs, suggesting that interactions between cells expressing IL-17 and IL-17R may be involved in the epileptogenic properties of FCDs. In addition, our result suggested that IL-17 exerts its biologic effects through the ACT1 and NFκB-p65 pathways in FCDs. Thus, pharmacologic modulation of the IL-17 signaling pathway may represent a potentially novel antiepileptic strategy in FCDs. Effective therapeutic intervention based on the modulation of the IL-17 system has to take into consideration the specific roles of IL-17, which require further investigation.

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REFERENCES


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43. Oby E, Janigro D. The blood-brain barrier and epilepsy. Epilepsy 2006;47:1761–74