Expression and Cellular Distribution of the Interleukin 2 Signaling System in Cortical Lesions From Patients With Focal Cortical Dysplasia

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Abstract

Focal cortical dysplasia (FCD) is a well-known cause of medically intractable epilepsy. To understand the potential role of the inflammatory cytokine interleukin 2 (IL-2) in the pathogenesis of FCD, we investigated the expression patterns of IL-2 and its receptors (IL-2Rs) in FCD and control samples that included epileptic neocortex from mesial temporal lobe epilepsy patients and nonepileptic normal cortex (CTX). Greater mRNA and protein levels of IL-2 and IL-2Rs were observed in FCD versus CTX samples. Moreover, the expression of IL-2 and IL-2Rs was significantly higher in FCD II than FCD I. In situ hybridization and immunohistochemistry results indicated that IL-2 and IL-2Rs were strongly expressed in hypertrophic neurons and neuronal microcolumns in FCD I and highly expressed in malformed cells in FCD II. In addition, the protein levels of Janus kinase 1, Janus kinase 3, phosphorylated signal transducer and activator of transcription 5, which are important downstream factors in the IL-2 signaling pathway, were increased in FCD lesions. Soluble IL-2R was decreased in FCD compared with that in CTX samples. These results suggest that upregulation of IL-2 and IL-2Rs combined with activation of IL-2-dependent signaling pathways may contribute to the pathogenesis of FCD.

Key Words: Astrocytes, Epilepsy, Focal cortical dysplasia, Inflammation, Interleukin 2, Malformations of cortical development, Neurons.

INTRODUCTION

Focal cortical dysplasia (FCD), which is characterized by sporadic architectural and cytoarchitectural malformations of the cerebral cortex, is the major cause of chronic medically intractable epilepsy in children (1). According to the current classification system, isolated forms of FCD can be distinguished into type I and type II (2). Previous studies have indicated that cortical lesions in FCD are likely to be epileptogenic foci (3), but the molecular mechanisms underlying the pathogenesis of FCD and associated epilepsy remain unclear.

The involvement of inflammatory processes in the pathophysiology of human epilepsy has received increasing attention (4, 5). The activation of both innate and adaptive immune responses and the upregulation of proinflammatory cytokine expression (i.e. interleukin 1β [IL-1β], IL-6, and tumor necrosis factor) have been described in human epileptogenic tissue, particularly in malformations of cortical development (1, 6, 7). Emerging evidence supports the hypothesis that activation of the inflammatory pathway may contribute to the generation and recurrence of seizures and seizure-related neuronal damage (8, 9). In addition to the innate immune response, several studies have demonstrated the presence of T cells in malformations of cortical development (1, 10). T cells may exert functional roles in mesial temporal lobe epilepsy (MTLE) (11, 12) and Rasmussen encephalitis (13), both of which are also associated with intractable epilepsy.

Interleukin 2, a cytokine also known as “T-cell growth factor,” exhibits a variety of biologic functions through its receptor complex and downstream signaling factors. Interleukin 2 receptors (IL-2Rs) are composed of 3 distinct subunits: IL-2Rα, IL-2Rβ, and IL-2Rγ. In different combinations, these subunits can form different functional IL-2R subtypes (14). Binding of IL-2 to the IL-2Rs mediates the activation of Janus kinase 1 (JAK1) and JAK3 (15); these kinases in turn result in the recruitment of the signal transducer and activator of transcription (STAT) (16). In addition, the soluble form of IL-2R (sIL-2R) is regarded as a marker of T-cell activation that can act by binding IL-2 to compete with cell-bound IL-2Rs (17).

In addition to multiple regulatory functions within the immune system, emerging evidence suggests that IL-2 is also a modulator of the CNS. Brain-derived IL-2 is potentially produced by neurons and astrocytes and is widely distributed throughout the brain (18). Alterations in brain-derived IL-2 and IL-2Rs have been implicated in the pathogenesis of several major neurologic disorders, including multiple sclerosis...
Intraventricular administration of IL-2 promotes seizures in various rodent models of epilepsy (21). Furthermore, high doses of IL-2 cause recurrent seizure activity (22); however, to date, no reports have investigated the expression of IL-2 and its receptors in FCD lesions.

Here, we analyzed the mRNA and protein expression of IL-2, with its receptors and downstream factors, in surgically resected FCD I and FCD II cortical lesions. We also investigated the specific cellular distribution of IL-2 and its receptors in both neuronal and glial components of FCD.

**MATERIALS AND METHODS**

**Subjects**

The specimens studied were obtained from the archives of the Department of Neurosurgery at Xinqiao Hospital (the Third Military Medical University in Chongqing, China). All procedures and experiments were approved by the Ethics Committee of the Third Military Medical University, where informed consent was obtained for the use of brain tissue and medical records for research purposes. All human specimens were obtained and used in a manner compliant with the Declaration of Helsinki. No tissue was resected solely for experimental purposes.

All patients underwent presurgical evaluation and met the criteria for epilepsy surgery. We examined surgical specimens from patients with FCD I (n = 14) and FCD II (n = 12). All cases were independently reviewed by 2 neuropathologists, and the diagnosis was confirmed according to the international consensus classification system of the International League Against Epilepsy (2). Detailed clinical data and applications performed on each patient specimen are listed in Table 1. Seizure duration represents the interval between the age at seizure onset and the age at surgery in years. The postoperative seizure outcome (Table 2) was assessed according to the criteria of Engel et al (23).

Samples from patients with MTLE, a common form of human intractable epilepsy, served as an epilepsy control group (n = 12). The detailed clinical data and applications performed on these patient samples are listed in Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A555.

Age-matched control cortex (CTX)/white matter samples were obtained from the autopsies of 9 patients (6 males/3 females; mean age, 6.0 years; range, 1–13 years) without a history of seizures or other neurologic diseases.

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*Patients with sufficient amount of perilesional zone for analysis.

F, female (sex); F, frontal lobe; FCD, focal cortical dysplasia; GTCS, generalized tonic-clonic seizure; IHC, immunohistochemistry (including immunofluorescence); IS, infantile spasm; ISH, in situ hybridization; M, male (sex); O, occipital lobe; P, parietal lobe; PO, postoperative outcome (Engel class); PS, partial seizure; real-time PCR, real-time quantitative polymerase chain reaction; T, temporal lobe; WB, Western blotting.
performed within 6 hours of death. Detailed data on the control patients are listed in Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A556. Most mRNAs and proteins, including the IL-2 family, are stable and therefore well preserved within 6 hours of death (24, 25). The autopsy cases were reviewed by 2 neuropathologists, and both gross and microscopic examinations showed no structural abnormalities. In addition, 5 patients (4 with FCD I and 1 with FCD II; Table 1) with a sufficient perilesional zone (normal-appearing cortex/white matter adjacent to the lesion) were examined. Specimens from the last group represent optimal control tissues because they were exposed to the same seizure activities, drugs, and fixation protocol.

**Tissue Preparation**

All the neocortical samples obtained at surgery or autopsy were immediately divided into 2 parts. One part was fixed in 10% buffered formalin and embedded in paraffin. Paraffin-embedded tissue was sectioned at 6 μm for histologic study, in situ hybridization (ISH), and immunohistochemistry (IHC). One section from every specimen was processed for IHC. One section from every specimen was processed for real-time polymerase chain reaction (PCR), Western blotting, and ELISA analyses.

**Real-time Quantitative PCR Analysis**

Real-time quantitative PCR analysis was performed using RNA prepared from freshly frozen histologically normal human cortex (n = 6) and specimens from patients with MTLE (n = 6) and FCD (type I and type II; n = 6 for each type). The RNA preparation contained equal proportions of gray and white matter tissues. Total RNA from each sample was extracted using Trizol reagent following the manufacturer’s instructions (Invitrogen, La Jolla, CA). The concentration and purity of RNA were determined spectrophotometrically at 260/280 nm with a nanodrop spectrophotometer (Ocean Optics, Dunedin, FL). One microgram of total RNA was reverse-transcribed into single-stranded complementary DNA with oligo (dT) primer (TakaRa, Otsu, Japan). Polymerase chain reaction primers were designed based on the complementary DNA sequence and synthesized by TaKaRa Biotechnology Company (Dalian, China). The following primers were used: IL-2 (forward: caaagaacagctactaggg, reverse: ttaaatgttagacctctggtga), IL-2Rα (forward: caggagacgcgtctacagc; reverse: cctctacctgcgaaactgctg), IL-2Rβ (forward: tagagttgtagaagacca; reverse: cggagtggagaaagaagtaa), IL-2Rγ (forward: tagatgagtgtgcctagggact; reverse: cagtagggacctctggtta), and β-actin (forward: gcaccacctttccaagtagc; reverse: tagacagctctgctgaacg). The cycling conditions for IL-2 were as follows, with any changes in the conditions used for the other primers indicated in parentheses: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing elongation at 65°C for 30 seconds (IL-2Rα 30 seconds at 58°C; IL-2Rβ 30 seconds at 58°C; IL-2Rγ at 58°C). The relative quantification of each product compared with the reference gene β-actin was evaluated by calculating \(2^{-\Delta\Delta Ct}\). All of the samples were run in triplicate, and the relative quantification of each target gene expression was performed twice.

**Antibody Characterization**

The primary antibodies used in this study are listed in Table, Supplemental Digital Content 3, http://links.lww.com/NEN/A557.

**Western Blotting**

Western blotting was performed to quantify the amount of IL-2, IL-2Rα, IL-2Rβ, and IL-2Rγ and downstream molecular proteins in MTLE and FCD lesions compared with CTX. Western blot analysis was performed on frozen specimens from histologically normal CTX (n = 12), MTLE (n = 14), FCD I (n = 14), and FCD II (n = 12) brain samples. The samples were homogenized in lysis buffer containing 150 mmol/L sodium chloride, 50 mmol/L Tris-HCl pH 7.5, 10% glycerol, 0.5% Triton X-100, 0.1% sodium dodecyl sulfate–polyacrylamide gel electrophoresis to differentiate by molecular weight and were then transferred onto polyvinylidene fluoride membranes. Membranes were blocked in 5% dry milk for 1 hour and incubated overnight with primary antibody. After washing and incubating with horseradish peroxidase–conjugated anti-rabbit or anti-mouse secondary antibodies (1:5000; Beyotime, Shanghai, China) for 1 hour at room temperature. Immunoreactivity (IR) was visualized using enhanced chemiluminescence. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was measured and used as a reference value.
**In Situ Hybridization**

Sections from tissue blocks were routinely stained with hematoxylin and eosin, and consecutive serial sections were used for ISH and IHC. In situ hybridization was assessed using commercial detection kits (Boster, Wuhan, China) with high-performance liquid chromatography purified oligonucleotide probes that were specific for IL-2 (catalog MK1162h), IL-2 Ro (catalog MK2075h), IL-2 Ry (catalog MK2077h) mRNA. Briefly, paraffin sections were deparaffinized, rehydrated, and incubated with 0.3% H2O2 for 30 minutes. After treatment with pepsin to expose mRNA (100 mg/mL in 3% citric acid, 30 minutes, 37°C), sections were prehybridized at 38°C for 3 hours and then hybridized with a digoxigenin-labeled oligonucleotide probe at 38°C overnight. After hybridization, the sections were washed in 2× SSC for 5 minutes at 37°C, 0.5× SSC for 15 minutes at 40°C, and 0.2× SSC for 15 minutes at 42°C. The immunoreactions were visualized using a ready-to-use SABC peroxidase system (Boster) with 3,3′-diaminobenzidine tetrahydrochloride hydrate (Boster) as the chromogen. Sections were counterstained with hematoxylin, dehydrated with alcohol, cleared with xylene, and placed on a coverslip. As negative controls, sections were incubated with a nonsense probe or PBS (omitting oligonucleotide probes).

**Immunohistochemistry**

Single-label IHC was performed using the avidin-biotin peroxidase staining method. The sections were incubated with primary antibodies overnight at 4°C. Thereafter, the sections were incubated with appropriate secondary antibodies (Boster) for 45 minutes at 37°C and then SABC peroxidase (Boster) for 30 minutes at 37°C. Immunoreactions were visualized with 3,3′-diaminobenzidine tetrahydrochloride hydrate. Sections were counterstained with hematoxylin, dehydrated, and coverslipped. No immunoreactive cells were detected in negative control experiments, which included application of the secondary antibody alone, preabsorption with a 10-fold excess of a specific blocking antigen, or incubation with an isotype-matched rabbit polyclonal antibody (Figure, Supplemental Digital Content 4, http://links.lww.com/NEA/A558).

For double immunofluorescence staining, sections were incubated with mixed primary antibodies overnight at 4°C. After rinsing, the sections were incubated with a mixture of CY5-conjugated anti-mouse antibody (1:500; Jackson Immuno-Research, West Grove, PA) and Alexa Fluor 488 anti-rabbit antibody (1:500; Molecular Probes, Eugene, OR) for 1 hour at 37°C. For negative controls, the primary antibodies were omitted. Counterstaining of cell nuclei was performed by incubating the sections with DAPI (Beyotime) for 25 minutes at room temperature. Fluorescent sections were observed and photographed using a laser scanning confocal microscope (Leica TCS-TIV, Nussloch, Germany). Images were converted to TIFF format, and contrast levels were adjusted using Adobe Photoshop 11.0 (Adobe Systems, San Jose, CA).

**ELISA**

The concentration of sIL-2R was measured by ELISA using commercial detection kits (Neobioscience Technology Company, Beijing, China). Histologically normal (n = 9), MTLE (n = 9), FCD I (n = 10), and FCD II (n = 10) frozen brain specimens were used for ELISA according to the manufacturer’s instructions. The sensitivity of the assays for sIL-2R was 16.0 pg/mL. All of the samples from epileptic patients and controls were equally distributed across several plates to minimize potential differences.

**Evaluation of Western Blot Analysis**

For the Western blot analysis, densitometric analysis was performed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The optical density values of each protein band were calculated and normalized to the optical density value of GAPDH.

**Evaluation of IHC and Cell Counting**

Evaluation of specific immunostaining and cell counting were performed by 2 independent observers. The overall concordance was greater than 90%, and the overall κ value ranged from 0.83 to 0.96. Interleukin 2 and IL-2 Ro/B/γ IR was evaluated using a Leica microscope as previously reported (26). Each section was divided into 200 high-power nonoverlapping fields using a square grid inserted into the eyepiece (0.0625 × 0.0625–mm width, each corresponding to 781.250 μm²). Staining intensity was evaluated using a semiquantitative staining intensity score (Table 3), calculated for each section and were calculated as an average across the selected fields (Table 3).

**Data Analysis and Statistics**

Data are expressed as the mean ± SE. SPSS for Windows (SPSS Inc., Chicago, IL) was used for the statistical analysis. Means were compared using analysis of variance (ANOVA), and differences between groups were considered significant for values of p < 0.05.

**RESULTS**

**Case Material and Histologic Features**

Normal-appearing cortical samples exhibited organized layers, with well-preserved laminations, unipolar orientation of apical dendrites toward the pial surface, and normal definition of the gray-white matter junction. None of the FCD cases were associated with other...
primary lesions (e.g., tumor or hippocampal sclerosis) and fulfilled the histopathologic criteria of FCD Ia or Iib (2). Focal cortical dysplasia type Ia is characterized by cortical dyslamination and the presence of microcolumns and HNs. Focal cortical dysplasia type Iib displays additional cytologic abnormalities, including DNs and BCs (Figure, Supplemental Digital Content 5, http://links.lww.com/NEN/A559). Hypertrophic neurons demonstrate a pyramidal morphology with a central nucleus. Dysmorphic neurons were defined as neurons with one or more eccentrically located nuclei.

Dysmorphic neurons with a thin membrane, eosinophilic cytoplasm, and Nissl substance. Balloon cells were identified as morphologically normal cells with a thin membrane, eosinophilic cytoplasm, and one or more eccentrically located nuclei.

### Interleukin 2 mRNA Expression

There was greater IL-2 mRNA expression in epilepsy groups compared with CTX (Fig. 1A). The IL-2 mRNA level was significantly higher in FCD II vs. FCD I. No significant differences were observed between MTLE and FCD (FCD I and FCD II) specimens. In situ hybridization analysis was performed to study the cellular distribution of IL-2 mRNA in CTX and FCD specimens. Histologically normal cortex displayed IL-2 mRNA expression in pyramidal neurons and glial cells (Fig. 1B). In FCD I, moderate to strong IL-2 mRNA expression was detected in HNs and in microcolumns (Fig. 1C). In FCD II there was strong IL-2 mRNA expression in MCs (Fig. 1D).

### IL-2 Western Blot and IHC Analysis

Consistent with the results obtained by real-time PCR, Western blot analysis showed increased IL-2 protein levels in the epilepsy group compared with CTX (Fig. 2A, B). The IL-2 protein level was significantly higher in FCD II versus those in FCD I and MTLE cases. In addition, IL-2 protein expression was higher in FCD I than those in MTLE cases. The distribution and cellular pattern of IL-2 IR in pyramidal neurons and glia in the CTX samples were similar to those in previous reports (Fig. 2C, F) (18). The immunostaining intensity scores indicated a higher expression of IL-2 in the MTLE and FCD specimens versus those in the CTX samples (Table 3).

In FCD I, IL-2 was intensely expressed in microcolumns (Fig. 2D) and in 79.7% ± 1.9% (n = 220) of HNs. Double labeling experiments revealed colocalization of IL-2 IR with NeuN in HNs and normal-appearing neurons (Fig. 2I). Interleukin 2 was coexpressed with GFAP in reactive astrocytes (Fig. 2J). In FCD II, 76.9% ± 2.6% of HNs (n = 470), 78.7% ± 2.4% of DNs (n = 560), and 70.0% ± 3.4% of BCs (n = 389) distributed from the pial surface to the subcortical white matter displayed IL-2 immunostaining (Fig. 2E, H). Interleukin 2 IR was also observed in normal-appearing neurons and cells exhibiting glial morphology in FCD lesions. Double labeling experiments confirmed colocalization of IL-2 IR with NeuN in MCs (Fig. 2K) and with GFAP in reactive astrocytes (Fig. 2L). In agreement with the abnormal differentiation of the BCs, we observed colocalization of IL-2 IR with vimentin in BCs (Fig. 2L insert). Further experiments revealed that T cells (CD3 positive) within the dysplastic cortex occasionally displayed IL-2 IR (Fig. 2N), but no HLA-DR-positive microglia in FCD lesions showed positive immunostaining of IL-2 (Fig. 2M).

### IL-2R mRNA Expression

No significant differences in IL-2Rα mRNA expression were observed between the CTX, MTLE, and FCD I specimens (Fig. 3A). Interleukin 2Rα levels were significantly higher in FCD II versus those in the other groups. There was greater IL-2Rβ and IL-2Rγ mRNA expression in the epilepsy groups compared with that in the CTX group. The IL-2Rβ mRNA level was significantly higher in FCD II and MTLE specimens versus those in FCD I specimens, and MTLE and FCD II were not significantly different. The IL-2Rγ mRNA

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**Table 3. Staining Scores for IL-2 and IL-2Rs in Control CTX, MTLE, FCD I, and FCD II**

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<td>0.36 ± 0.16</td>
<td>1.38 ± 0.14**</td>
<td>1.29 ± 0.24**</td>
<td>1.94 ± 0.27**</td>
</tr>
<tr>
<td>IL-2Rα</td>
<td>0.43 ± 0.17</td>
<td>1.92 ± 0.18**</td>
<td>1.57 ± 0.25**</td>
<td>1.75 ± 0.26**</td>
</tr>
<tr>
<td>IL-2Rβ</td>
<td>1.07 ± 0.20</td>
<td>1.83 ± 0.22*</td>
<td>1.86 ± 0.21*</td>
<td>2.33 ± 0.17**</td>
</tr>
<tr>
<td>IL-2Rγ</td>
<td>0.64 ± 0.20</td>
<td>1.58 ± 0.21**</td>
<td>1.71 ± 0.22**</td>
<td>2.09 ± 0.24**</td>
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Data are expressed as mean ± SE. *p < 0.05, **p < 0.01. MTLE, FCD I, FCDII versus CTX. Analysis of variance.

**Table 4. Labeling Indices of IL-2 and IL-2R Immunoreactivity in FCD I and FCD II**

<table>
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<th>Antibody to</th>
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<td>IL-2</td>
<td>79.7% ± 1.9%</td>
<td>70.0% ± 3.4%</td>
<td>78.7% ± 2.4%</td>
<td>76.9% ± 2.6%</td>
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<tr>
<td>IL-2Rα</td>
<td>80.8% ± 1.6%</td>
<td>75.1% ± 3.0%</td>
<td>81.8% ± 3.6%</td>
<td>73.6% ± 2.3%</td>
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<tr>
<td>IL-2Rβ</td>
<td>81.9% ± 1.4%</td>
<td>75.9% ± 3.5%</td>
<td>82.9% ± 2.1%</td>
<td>81.1% ± 1.6%</td>
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<tr>
<td>IL-2Rγ</td>
<td>80.6% ± 1.9%</td>
<td>72.6% ± 3.4%</td>
<td>80.4% ± 2.4%</td>
<td>78.6% ± 2.6%</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. BCs, balloon cells; DN, dysmorphic neurons; FCD, focal cortical dysplasia types I and II; HNs, hypertrophic neurons; IL-2, interleukin 2; IL-2Rα, -β, -γ, interleukin 2 receptor-α, -β, -γ.
level was significantly higher in FCD II versus those in the MTLE and FCD I specimens, and MTLE and FCD I were not significantly different.

In situ hybridization analysis showed the cellular distribution of IL-2Rα, IL-2Rβ, and IL-2Rγ mRNA in CTX and FCD samples. Weak to modest IL-2R subunit mRNA expression was observed in pyramidal neurons and glial cells in CTX (Fig. 3B, E, H). In FCD I, there was moderate to strong IL-2Rα, IL-2Rβ, and IL-2Rγ mRNA expression in HNs and microcolumns (Fig. 3C, F, I). In FCD II, there was strong IL-2Rα, IL-2Rβ, and IL-2Rγ mRNA expression in MCs (Fig. 3D, G, J).

IL-2R Western Blot and IHC

Larger IL-2Rα protein levels were obtained by Western blot analysis in the epilepsy groups compared with those in the CTX group (Fig. 4A, B). The IL-2Rα protein level was significantly higher in MTLE versus those in FCD specimens. No significant difference was observed between FCD I and FCD II specimens. The IL-2Rβ protein level was significantly higher in FCD II versus those in the other groups. Greater IL-2Rβ protein expression was detected in MTLE compared with CTX and FCD I, and CTX and FCD I were not significantly different. The IL-2Rγ protein level was significantly higher in FCD than those in CTX and MTLE samples. Increased IL-2Rγ protein expression was detected in FCD II compared with that in FCD I, and no significant difference was observed between CTX and MTLE specimens. Weak to moderate IL-2γ staining was detected in neurons and glial cells in CTX samples (Figs. 4C, F; 5A, D; 6A, D). The cellular pattern of IL-2Rs in normal cortex was consistent with those in previous reports (27). The intensity scores of IL-2Rα, IL-2Rβ, and IL-2Rγ IR in the MTLE and FCD specimens were dramatically higher than those in the CTX samples (Table 3). In MTLE specimens, there was moderate to strong IL-2 staining detected in neurons and glial cells (Fig. 7A). Double labeling experiments demonstrated colocalization of IL-2 IR with NeuN in neurons (Fig. 7B) and with GFAP in reactive astrocytes (Fig. 7C). Moderate to strong IL-2Rα, IL-2Rβ, and IL-2Rγ staining was detected in neurons and glial cells in MTLE specimens (Fig. 7D, G, J). Double labeling experiments demonstrated colocalization of IL-2Rα, IL-2Rβ, and
**FIGURE 2.** Interleukin-2 (IL-2) protein expression in control cortex (CTX) and epilepsy specimens. (A, B) Representative immunoblot bands (A) and densitometric analysis (B) of total homogenates from control cortex, mesial temporal lobe epilepsy (MTLE), focal cortical dysplasia type I (FCD I), and FCD II specimens. The expression of the internal control protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is shown in the same protein extracts. Relative optical densities (ODs) of the bands were obtained from 3 independent experiments. Data are expressed as the mean ± SE. **p < 0.01. Analysis of variance. (C–H) Cell-specific distribution of IL-2 in CTX, FCD I, and FCD II specimens. Histologically normal cortex shows weak to moderate IL-2 immunoreactivity (IR) in neurons (arrows and insert in C) and glia-like cells (arrowheads and insert in F). In FCD I, there is moderate to strong IL-2 IR in microcolumns (double arrows and insert in D), heterotopic neurons (arrows and insert in G), and glia-like cells (arrowheads and insert in G). In FCD II (E and H), there is strong IL-2 IR in malformed cells (MCs), including dysmorphic neurons (DNs) (arrowheads), hypertrophic neurons (HNs) (double arrows), and reactive astrocytes (double arrowheads). High-magnification view shows strong IL-2 expression in balloon cells (BCs) (insert a in E, arrows), DNs (insert b in E, arrowheads), HNs (insert c in E, double arrow), and reactive astrocytes (insert in H, double arrowheads). (I–N) Confocal images show the colocalization of IL-2 (green) with NeuN (red) in neurons (arrows) (I) and the colocalization of IL-2 (green) with glial fibrillary acidic protein (GFAP) (red) in reactive astrocytes (J, insert, arrowheads) but not in neurons (arrows). (K) IL-2–positive (green) DNs (arrows) and HNs (arrowheads) colabeled with NeuN (red). (L) No IL-2–positive (green) MCs (arrows) colocalized with GFAP (red), but IL-2–positive reactive astrocytes (arrowheads) colocalized with GFAP. Colocalization of IL-2 (green) with vimentin (red) in BCs (insert in L). (M) No IL-2–positive (green) cells (arrows) colabeled with HLA-DR (red). (N) IL-2–positive (green) T cells (arrows) colabeled with CD3 (red). Sections were counterstained with hematoxylin (C–H) or DAPI (I–N). Scale bars = (C–I) 50 μm; (J–L, inserts in C–H) 20 μm; (M, N, inserts in J, L) 10 μm.
IL-2Rγ IR with NeuN in neurons (Fig. 7E, H, K) and with GFAP in reactive astrocytes (Fig. 7F, I, L).

In FCD I, moderate to strong IL-2Rα, IL-2Rβ, and IL-2Rγ staining was detected in microcolumns (Figs. 4D, 5B, 6B) and 80.8% ± 1.6% (n = 179), 81.9% ± 1.4% (n = 301), and 80.6% ± 1.9% (n = 233) of HNs, respectively. Double labeling experiments revealed colocalization of IL-2α, IL-2β, and IL-2γ IR with NeuN in HNs and normal-appearing neurons (Figs. 4I, 5G, 6G). Interleukin 2α, IL-2β, and IL-2γ IR were coexpressed with GFAP in reactive astrocytes (Figs. 4I, 5H, 6H).

In FCD II, 73.6% ± 2.3% of HNs (n = 505), 81.8% ± 3.6% of DNs (n = 511), and 75.1% ± 3.0% of BCs (n = 330) displayed IL-2Rα staining; 81.1% ± 1.6% of HNs (n = 533), 82.9% ± 1.4% of DNs (n = 600), and 79.5% ± 3.5% of BCs (n = 410) displayed IL-2Rβ staining; and 78.6% ± 2.6% of HNs (n = 410), 80.4% ± 2.4% of DNs (n = 460), and 72.6% ± 3.4% of BCs (n = 313) displayed IL-2Rγ staining throughout the cortex and white matter (Figs. 4E, H; 5C, F; 6C, F). Interleukin 2α, IL-2β, and IL-2γ IR was also observed in normal-appearing neurons and cells exhibiting glial morphology in FCD lesions. Double labeling experiments confirmed colocalization of IL-2Rα, IL-2Rβ, and IL-2Rγ IR with NeuN in MCs (Fig. 4K, 5I, 6I) and with GFAP in reactive astrocytes (Figs. 4L, 5J, 6J). Interleukin 2α, IL-2β, and IL-2γ IR colocalized with vimentin in BCs (Figs. 4N, 5L, 6L). CD3-positive T cells within the dysplastic cortex occasionally displayed IL-2Rα, IL-2Rβ, and IL-2Rγ IR (Figs. 4M, 5K, 6K), but no HLA-DR-positive microglia in FCD lesions showed positive immunostaining for IL-2Rα, IL-2Rβ, and IL-2Rγ (data not shown).

**JAK1, JAK3, and p-STAT5 Expression**

Interleukin 2 mediates the activation of several major signaling pathways, including the Janus kinase–signal transducer and activator of transcription (JAK/STAT) pathway. Janus kinase 1 and JAK3 in turn mediate the activation of IL-2 and IL-2R signaling; 81.1% ± 1.6% of HNs (n = 533), 82.9% ± 1.4% of DNs (n = 600), and 79.5% ± 3.5% of BCs (n = 410) displayed IL-2Rβ staining; and 78.6% ± 2.6% of HNs (n = 410), 80.4% ± 2.4% of DNs (n = 460), and 72.6% ± 3.4% of BCs (n = 313) displayed IL-2Rγ staining throughout the cortex and white matter (Figs. 4E, H; 5C, F; 6C, F).

**Soluble IL-2 ELISA**

Soluble IL-2 levels in FCD specimens were lower than those in CTX samples (FCD I vs CTX, p < 0.05; FCD II vs CTX, p < 0.05) (Fig. 9). In contrast, the sIL-2R level in MTLE was slightly lower than those in CTX samples (Fig. 9), but this difference was not significant (p > 0.05).

**DISCUSSION**

The involvement of inflammatory processes in the pathophysiology of human epilepsy has received increased attention in recent years. Interleukin 2 is a cytokine that is mainly produced by activated T cells, and it has multiple immunoregulatory functions that are related to T cells and the CNS (18, 29). In the present study, upregulated expression patterns of IL-2 and its receptors were detected in MTLE and cortical lesions of FCD compared with those in histologically normal CTX samples at both the mRNA and protein levels. Intriguingly, ISH and IHC results demonstrated that the high-level expression of mRNA and protein, including IL-2 and its receptors, was mainly localized within microcolumns and MCs, including HNs, DNs, and BCs. In addition, the protein levels of important downstream factors in the IL-2 pathway, JAK1, JAK3, and p-STAT-5, were significantly increased in MTLE and FCD specimens versus those in CTX samples. This advantage of the use of CTX samples from postmortem brains is autolysis. Thus, the use of high-quality samples from rapidly performed autopsies (i.e. within 6 hours of death) was important in the present study.

**IL-2 and IL-2Rs in CTX**

Previous studies have demonstrated IL-2 and IL-2Rs in human (30, 31) and rodent brains (32). In histologically normal brains, the IL-2/IL-2R system was localized in many regions, with neurons and glial cells being potential sources (33). In the present study, we detected IL-2 and IL-2Rα, IL-2Rβ, and IL-2Rγ mRNA and protein expression in FCD II samples using real-time PCR and Western blotting. In situ

![FIGURE 3](image-url) IL-2Rα, IL-2Rβ, and IL-2Rγ mRNA expression in control cortex (CTX) and epilepsy specimens. (A) Real-time PCR of IL-2 mRNA expression in control cortex (CTX) and epilepsy specimens. (B) IHC staining for IL-2 in control cortex (CTX) and epilepsy specimens. (C) IHC staining for IL-2Rα in control cortex (CTX) and epilepsy specimens. (D) IHC staining for IL-2Rβ in control cortex (CTX) and epilepsy specimens. (E) IHC staining for IL-2Rγ in control cortex (CTX) and epilepsy specimens.
FIGURE 4. Interleukin 2 receptor-α, IL-2Rβ, and IL-2Rγ protein expression in control cortex (CTX) and epilepsy specimens. (A, B) Representative immunoblot bands (A) and densitometric analysis (B) of total homogenates from control CTX, mesial temporal lobe epilepsy (MTLE), focal cortical dysplasia type I (FCD I), and FCD II specimens. Expression of the internal control protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is shown in the same protein extracts. Relative optical densities (ODs) of the bands were obtained from 3 independent experiments. Data are expressed as mean ± SE. *p < 0.05, **p < 0.01. Analysis of variance. (C–N) Cell-specific distribution of IL-2Rα in CTX, FCD I, and FCD II specimens. (C, F) In normal CTX, there is weak to moderate IL-2Rα immunoreactivity (IR) in neurons (arrows and insert in C) and glia-like cells (arrowheads and insert in F). In FCD I, there is moderate to strong IL-2Rα IR in microcolumns (double arrows and insert in D), heterotopic neurons (arrows and insert in G), and glia-like cells (arrowheads and insert in G). (E, H) In FCD II, there are variable appearances of IL-2Rα IR. There is strong IL-2Rα IR in malformed cells (MCs), including balloon cells (BCs) (insert a in E, arrows), dysmorphic neurons (DNs) (arrowheads and insert b in E), hypertrophic neurons (HNs) (double arrows and insert c in E), and reactive astrocytes (double arrowheads and insert in H). (I) In FCD I, confocal images show the colocalization of IL-2Rα (green) with NeuN (red) in neurons (arrows) and the colocalization of IL-2Rα (green) with glial fibrillary acidic protein (GFAP) (red) in reactive astrocytes (arrowheads) but not in neurons (arrows). (K) IL-2Rα-positive (green) DNs (arrows) and normal-appearing neurons (arrowheads) are colabeled with NeuN (red). (L) No IL-2Rα-positive (green) MCs (arrows) colocalized with GFAP (red), but IL-2Rα-positive reactive astrocytes (arrowheads and insert) colocalized with GFAP. (M) IL-2Rα-positive (green) T cells (arrows) colabeled with CD3 (red). (N) Co-localization of IL-2Rα (green) with vimentin (red) in BCs (arrows) but not in DNs (arrowheads). Sections were counterstained with hematoxylin (C–H) or DAPI (I–N). Scale bars = (C–H) 50 μm; (I–N, inserts in C–G) 20 μm; (inserts in H, L) 10 μm.
hybridization and IHC results revealed weak to moderate IL-2/IL-2R expression in pyramidal neurons and glial cells in these samples.

**Enhanced Expression of IL-2 in FCD**

The IL-2 signaling system has been implicated in several neurologic disorders, including hypoxia-ischemia brain injury (32), Tourette syndrome (24), multiple sclerosis (19, 34), white matter damage (20), and Parkinson disease (30), but little is known about the cellular sources of IL-2 detected in these brains. In the present study, we detected greater IL-2 mRNA and protein levels in tissue homogenates of FCD versus those in CTX specimens. In addition, ISH and IHC results displayed high levels of IL-2 expression in MCs, microcolumns, and normal-appearing neurons in FCD lesions, indicating that these cells could be the sources of IL-2.
Although a previous study suggested that microglia might be the source and target of IL-2 (35), we did not detect any microglia in FCD lesions showing IL-2 IR. In contrast, we detected IL-2 in T cells and reactive astrocytes, indicating that brain-infiltrating T cells and reactive astrocytes could be sources of IL-2.

To our knowledge, the induction of IL-2 by seizures has not previously been demonstrated, but the effect of IL-2 on seizures has been previously assessed. Intraventricular administration of IL-2 facilitates seizures in rodent models by decreasing the latency to epileptiform discharges and increasing the duration of seizure activity (21, 22). Furthermore, high doses of IL-2 could cause recurrent seizure activity (5). These results suggest that the increased IL-2 levels in the epilepsy group may have contributed to the intrinsic and high epileptogenicity of FCD and MTLE. Interestingly, plasma and

**FIGURE 6.** Cell-specific distribution of interleukin 2 receptor-γ (IL-2Rγ) in control cortex (CTX), focal cortical dysplasia type I (FCD I), and FCD II. (A, D) Weak to moderate IL-2Rγ immunoreactivity (IR) in neurons (arrows and insert in A) and glia-like cells (arrowheads and insert in D) in histologically normal cortex. (B, E) IL-2Rγ IR in FCD I. There is moderate to strong IL-2Rγ IR in microcolumns (double arrows and insert in B), heterotopic neurons (arrows and insert in E), and glia-like cells (arrowheads and insert in E). (C, F) IL-2Rγ IR in FCD II. There are variable appearances of strong IL-2Rγ IR in balloon cells (BCs) (arrows and insert a in C), dysmorphic neurons (DNs) (arrowheads and insert b in C), hypertrophic neurons (double arrows and insert c in C), and reactive astrocytes (double arrowheads and insert in F). (G, H) Confocal images show the colocalization of IL-2Rγ (green) with NeuN (red) in neurons (arrows) (G) and the colocalization of IL-2Rγ (green) with glial fibrillary acidic protein (GFAP, red) in reactive astrocytes (arrowheads) (H). (I) IL-2Rγ-positive (green) DNs (arrows) and normal-appearing neurons (arrowheads) colabeled with NeuN (red). (J) No IL-2Rγ–positive (green) malformed cells (arrows) colocalized with GFAP (red), but IL-2Rγ–positive reactive astrocytes (arrowheads) colocalized with GFAP. (K) IL-2Rγ–positive (green) T cells (arrows) colabeled with CD3 (red). (L) Colocalization of IL-2Rγ (green) with vimentin (red) in BCs. Sections were counterstained with hematoxylin (A–F) or DAPI (G–L). Scale bars = (A–G, I) 50 μm; (H, J, K, L, insert in A–F) 20μm.
FIGURE 7. Cell-specific distribution of interleukin 2 (IL-2) and IL-2 receptors (IL-2Rα, IL-2Rβ, and IL-2Rγ) in mesial temporal lobe epilepsy (MTLE). (A) Moderate to strong IL-2 immunoreactivity (IR) in neurons (arrows and insert) and glia-like cells (arrowheads) in MTLE. (B) Merged images show the colocalization of IL-2 (green) with NeuN (red) in neurons (arrows). (C) Colocalization of IL-2 (green) with glial fibrillary acidic protein (GFAP) (red) in reactive astrocytes (arrowheads) but not in neurons (arrows). (D) Moderate to strong IL-2Rα IR in neurons (arrows and insert) and glia-like cells (arrowheads and insert). (E, F) Confocal images show that IL-2Rα-positive (green) neurons (arrows) colocalized with NeuN (red) (E). IL-2Rα-positive reactive astrocytes (arrowheads) colocalized with GFAP (red) (F). (G) Moderate to strong IL-2Rβ IR in neurons (arrows and insert) and glia-like cells (arrowheads). (H, I) Merged images show the colocalization of IL-2Rβ (green) with NeuN (red) in neurons (arrows) (H) and colocalization of IL-2Rβ (green) with GFAP (red) in reactive astrocytes (arrowheads) but not in neurons (arrows) (I). (J) Moderate to strong IL-2Rγ IR in neurons (arrows and insert) and glia-like cells (arrowheads) in MTLE. (K, L) Double labeling staining shows the colocalization of IL-2Rγ (green) with NeuN (red) in neurons (arrows) (K) and colocalization of IL-2Rγ (green) with GFAP (red) in reactive astrocytes (arrowheads) but not in neurons (arrows) (L). Sections were counterstained with hematoxylin (A, D, G, J) or DAPI (B, C, E, F, H, I, K, L). Scale bars = (A, D, G, J) 50 μm; (B, C, E, F, H, I, K, L, inserts in A, D, G, J) 20 μm.
FIGURE 8. Western blotting analysis of Janus kinase 1 (JAK1), JAK3, and p-STAT5 protein levels. (A, B) Representative immunoblot bands (A) and densitometric analysis (B) of total homogenates from control cortex (CTX), mesial temporal lobe epilepsy (MTLE), focal cortical dysplasia type I (FCD I), and FCD II specimens. Expression of the internal control protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is shown for the same homogenate samples. Relative optical densities (OD) of the bands were obtained from 3 independent experiments. Data are expressed as the mean ± SE. * p < 0.05, ** p < 0.01. Analysis of variance.
IL-2 Signaling System in Focal Cortical Dysplasia

Cerebrospinal fluid IL-2 levels were unchanged in patients with prolonged febrile seizures (36), suggesting that seizures alone might not account for the increased IL-2 expression in FCD and MTLE. Therefore, the lesion per se or the coexistence of the lesion and seizure activity likely results in modulation of the IL-2 system in FCD and MTLE.

Enhanced Expression of IL-2 Receptors in FCD

Interleukin 2Rβ and IL-2Rγ are critical for signal transduction, whereas IL-2Rα augments binding affinity but does not contribute to IL-2 signal transduction (16). We detected greater mRNA and protein levels of IL-2Rα, IL-2Rβ, and IL-2Rγ in cortical lesions of FCD patients. Interleukin 2Rα, IL-2Rβ, and IL-2Rγ are mainly located in MCs and T cells. The similar expression patterns for IL-2 and IL-2Rα, IL-2Rβ, and IL-2Rγ suggest that IL-2 may function through an autocrine/paracrine mechanism on MCs and T cells.

The neuroprotective effect mediated by IL-2 has been well recognized. Interleukin 2 enhances the viability and survival of cortical neurons (37, 38). Moreover, IL-2 significantly promotes the elongation and branching of neurites in hippocampal neurons (39). These data support the hypothesis that the IL-2 signaling system may protect MCs from cell death in the cortical lesion of FCD through an autocrine/paracrine mechanism.

Interleukin 2 exhibits a variety of functions related to T-cell migration, proliferation, and survival; it has long been recognized as a chemoattractant for T cells (40). Interleukin 2 can upregulate the expression of CC chemokine receptors and adhesion molecules that mediate the recruitment of activated T cells and their infiltration into tissues (41). More importantly, intracerebroventricular administration of IL-2 led to T-cell invasion of the brain (42). In addition, continued exposure to IL-2 prevented apoptosis in activated T cells by upregulating Bcl-2 protein (43). These results suggest that the infiltrating T cells could be a target of IL-2, which may modulate chemotaxis and the survival of these T cells, thereby contributing to the inflammatory state of the epileptic brain.

Soluble IL-2R

Lower sIL-2R levels were detected in FCD compared with those in CTX samples. A slightly, but not significantly, reduced sIL-2R level was found in MTLE versus that in CTX. Soluble IL-2R may compete with membrane-bound receptors for IL-2 and thus exert an antagonist effect on the IL-2 signal. We speculate that decreased sIL-2R concentrations likely reinforce the bioavailability of IL-2, and that this might represent an underlying mechanism of pathogenesis in FCD.

JAK-STAT Pathway

It has been well established that IL-2 could be responsible for activation of 3 major signaling pathways, including the JAK/STAT, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase pathways. Attention has been focused on activation of the phosphatidylinositol 3-kinase (44, 45) and mitogen-activated protein kinase (46) signaling pathways, both of which likely contribute to the pathogenesis and histopathologic features of malformations of cortical development. Therefore, to determine whether IL-2 signaling is mediated via the IL-2Rs/JAK/STAT pathway, we analyzed the changes in JAK1, JAK3, and p-STAT5 expression in MTLE and FCD relative to those in CTX samples. Markedly greater JAK1, JAK3, and p-STAT5 protein levels were detected in MTLE and FCD versus those in CTX specimens, indicating that activation of the IL-2Rs/JAK/STAT pathway could potentially be involved in IL-2–dependent pathogenesis of MTLE and FCD. However, it should be noted that IL-2Rβ is shared by IL-2 and IL-15 and that the IL-2Rγ is a common subunit of receptor complexes for all of the IL-2 family cytokines (including IL-4, IL-7, IL-9, IL-15, and IL-21) (47). To date, no reports have investigated the expression of these cytokines in FCD, which might also trigger the activation of the IL-2Rs/JAK/STAT pathway in FCD lesions. Therefore, further investigation is required to clarify the direct contribution of IL-2 to the activation of the IL-2Rs/JAK/STAT pathway in FCD lesions.

The following limitations should be noted in interpreting our results. First, age-unmatched MTLE specimens were used as a control group. We were apprehensive that the age and/or the seizure duration might influence the expression of IL-2. Thus, we investigated the mRNA and protein levels of IL-2 in pediatric MTLE specimens (n = 5; mean age, 9.2 years; range, 7–13 years; Table, Supplemental Digital Content 6, http://links.lww.com/NEN/A560), and no difference was detected compared with those in adult MTLE specimens (Figure, Supplemental Digital Content 7, http://links.lww.com/NEN/A561). Second, we observed differences between mRNA and protein expressions of IL-2 and receptors. The reasons for these differences remain unclear, but we assume that it might in part be caused by the regulatory...
mechanisms of mRNA transcription and protein translation. Third, the present study only includes specimens from FCD Ia and FCD IIb patients; therefore, the issue of whether the induction of IL-2 and its receptor expression also occurs in FCD IIb and FCD Ila still needs to be clarified.

In conclusion, our findings demonstrate a strong association between overproduction and overexpression in the IL-2 signaling system and FCDs. The high expression of IL-2 signaling in microcolumns and various MCs indicates that different cellular components of dysplastic cortex are involved in the IL-2 signaling system. The underlying mechanisms of IL-2 signaling system in the pathogenesis of FCDs could not be elucidated because of the limitations of a human study. Further studies are required to evaluate the biologic significance of altered IL-2 signaling system expression and distribution within the dysplastic cortex.

ACKNOWLEDGMENTS

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