Molecular alterations in epilepsy-associated malformations of cortical development
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The IL-1β system in epilepsy-associated malformations of cortical development

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ABSTRACT

Focal cortical dysplasia (FCD) and glioneuronal tumors (GNTs) are recognized causes of chronic intractable epilepsy. The cellular mechanism(s) underlying their epileptogenicity remains largely unknown. Compelling evidence in experimental models of seizures indicates an important role of interleukin (IL)-1β in the mechanisms of hyperexcitability leading to the occurrence of seizures. We investigated immunocytochemically the brain expression and cellular distribution pattern of IL-1β, IL-1 receptor (IL-1R) types I and II, and IL-1R antagonist (IL-1Ra) in FCD and GNT specimens, and we correlate these parameters with the clinical history of epilepsy in patients with medically intractable seizures. In normal control cortex, and in perilesional regions with histologically normal cortex, IL-1β, IL-1Rs and IL-1Ra expression was undetectable. In all FCD and GNT specimens, IL-1β and its signaling receptor IL-1RI were highly expressed by more than 30% of neurons and glia, whereas the decoy receptor IL-1RII and IL-1Ra were expressed to a lesser extent by ~10% and 20% of cells, respectively. These findings show a high expression of IL-1β and its functional receptor (IL-1RII) in FCD and GNT specimens together with a relative paucity of mechanisms (IL-1RII and IL-1Ra) apt to inactivate IL-1β actions. Moreover, the number of IL-1β- and IL-1RII-positive neurons was positively correlated with the frequency of seizures, whereas the number of IL-1Ra-positive neurons and astroglial cells was negatively correlated with the duration of epilepsy prior to surgery. The expression of IL-1β family members in these developmental lesions may contribute to their intrinsic and high epileptogenicity, thus possibly representing a novel target for antiepileptic strategies.

INTRODUCTION

Malformative and neoplastic glioneuronal lesions are a major cause of pediatric epilepsy [29, 69, 205, 234]. Although focal cortical dysplasia (FCD) and glioneuronal tumors (GNTs) are two distinct neuropathological entities, both are characterized by the presence of dysplastic neurons, activated or tumor (GNT) glial cells and phenotypically undefined glioneuronal elements [69]. A recent classification scheme for malformations of cortical development includes FCD and GNT among the disorders of proliferation (with abnormal cell types) together with the tuberous sclerosis complex and hemimegalencephaly [13]. Moreover, recent evidence indicates that epilepsy-associated glioneuronal lesions share common pathogenetic mechanisms [67]. Several reports suggest that both types of lesion (FCD and GNT) are intrinsically epileptogenic [29, 71, 235-238]; however the underlying cellular mechanism(s) remains largely unknown. In this respect, an important role in hyperexcitability has been attributed to the neuronal component of these lesions, consisting of highly differentiated cells containing, e.g., neuropeptides, neurotrophins, gap-junctions and receptors for different neurotransmitters [29, 69, 71, 111, 113, 239, 240]. Neuron-glia interactions may also play a critical role in the generation of seizures. Accordingly, several studies demonstrate alterations of functional properties of glial cells, involving plasma membrane channels and receptors that might be involved in epileptogenesis (for review, see [68]). A novel hypothesis is that the presence of activated astrocytes and microglia in FCD and GNT may be related to the epileptogenicity of these lesions through the production of...
inflammatory cytokines. Thus, an increasing number of observations in experimental models of seizures support the role of inflammatory molecules in ictogenesis and epileptogenesis (reviewed in [82, 84, 95, 241]). Particular attention has been focused on the role of the interleukin (IL)-1β activated pathways in ictogenesis. Intracerebral application of IL-1β in rodents prolongs seizure activity [93]. Accordingly, intracerebral application of the naturally occurring antagonist of the IL-1 receptor (IL-1Ra) mediates powerful anticonvulsant effects [94, 96, 241]. The threshold for induction of febrile seizures in immature rodents is decreased by IL-1β, whereas it is increased by IL-1Ra and in IL-1 receptor type 1 (IL-1RI)-deficient mice [91, 92]. Inhibition of the production of the biologically active form of IL-1β using blockers of interleukin-converting enzyme, also named caspase-1, significantly reduces seizures in rodents [97]. These observations strongly support a proconvulsant role of IL-1β produced in the brain in pathological conditions, such as during seizures or as a consequence of underlying inflammatory processes [92-94, 96, 242-245].

Studies in human tissue from epileptic patients, although still limited, support the experimental findings obtained in rodents. In particular, increased levels of proinflammatory molecules have been found in the cerebral spinal fluid and serum from epileptic patients [101-103]. Moreover, increased expression of inflammatory genes and related proteins has been observed in the brain tissue of patients with tuberous sclerosis complex and Rasmussen encephalitis, two neurological conditions associated with epilepsy [107, 246]. It has also been shown that the density of activated microglia (one major source of cytokines in the brain) in GNTs and FCD correlates with both the duration of epilepsy and the frequency of seizures [108, 109]. Finally, genetic studies reported a polymorphism in the promoter region of the IL-1β gene, which is associated with therapy-resistant temporal lobe epilepsy and febrile seizures [247-249]. In the present histological study, we demonstrate the intralesional expression and cellular distribution of IL-1β, IL-1 receptors and IL-1Ra in FCD and GNT specimens from patients with medically intractable epilepsy, thus providing direct evidence of a chronic inflammatory state in epileptogenic brain lesions. The presence of IL-1β-, IL-1RI- and IL-1Ra-positive cells is associated with the clinical course of epilepsy in patients. This study, together with functional and pharmacological evidence in experimental models, supports the role of the IL-1β system in the mechanisms underlying the intrinsic and high epileptogenicity of malformative and neoplastic glioneuronal lesions.

**MATERIALS AND METHODS**

**Subjects**
The cases included in this study were obtained from the files of the Departments of Neuropathology of the Academic Medical Center (University of Amsterdam) and the University Medical Center in Utrecht. We examined a total of 27 specimens removed from patients undergoing surgery for severe FCD (n=9), or GNTs (ganglioglioma, GG; n=9 and dysembryoplastic neuroepithelial tumor, DNT; n=9). Informed consent was obtained for the use of brain tissue and for access to medical records for research purposes. Tissue was obtained and used in a manner compliant with the Declaration of Helsinki. Two neuropathologists reviewed all cases independently and confirmed the diagnosis of FCD or GNT according to the revised WHO classification of tumors of the nervous system [250]. For grading the degree of FCD, we followed the classification system proposed by Palmini et
al. [15]. Normal-appearing control cortex/white matter from frontal, temporal and occipital regions was obtained at autopsy from 6 individuals (male/female: 3/3; mean age 27, range 4-55) without history of seizures or other neurological diseases. All autopsies were performed within 12 hours after death. We also selected 6 cases (3 FCD and 3 GG) that contained sufficient amount of perilesional zone (normal-appearing cortex/white matter adjacent to the lesion), for comparison with the autopsy specimens. Normal tissue adjacent to the lesional zone represents good disease control tissue because it is exposed to the same seizure activity, drugs, fixation time, and age and gender are the same. Table 1 summarizes the clinical features (derived from the patient medical records) with particular attention to the characteristics of seizures (type and frequency of seizures, age at seizure onset and postoperative seizure outcome). The predominant type of seizure pattern was that of complex partial seizures, which were resistant to maximal tolerated doses of antiepileptic drugs. Information concerning the exact time of last seizure occurrence prior to surgical resection was not available. However, all the patients included in our series did not have seizure activity in the last 24 h before surgery.

Table 1. Summary of clinical features of epilepsy patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Focal cortical dysplasia (FCD; n=9)</th>
<th>Ganglioglioma (GG; n=9)</th>
<th>Dysembryoplastic neuroepithelial tumor (DNT; n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>5/4</td>
<td>5/4</td>
<td>4/5</td>
</tr>
<tr>
<td>Mean age at surgery (years)</td>
<td>13.2 (4 - 31)</td>
<td>34.5 (10 - 56)</td>
<td>27.2 (5 - 40)</td>
</tr>
<tr>
<td>Location</td>
<td>Frontal: 1; Temporal: 8</td>
<td>Frontal: 1; Temporal: 8</td>
<td>Frontal: 1; Temporal: 8</td>
</tr>
<tr>
<td>Seizure type</td>
<td>CPS (100%); SGS (44%)</td>
<td>CPS (100%); SGS (33%)</td>
<td>CPS (100%); SGS (33%)</td>
</tr>
<tr>
<td>Mean age at seizure onset (years)</td>
<td>2.9 (0.6 - 15)</td>
<td>20.3 (2 - 30)</td>
<td>15.5 (1 - 30)</td>
</tr>
<tr>
<td>Duration of epilepsy (years)</td>
<td>10.2 (3 - 18)</td>
<td>15.3 (1 - 36)</td>
<td>11.6 (1 - 19)</td>
</tr>
<tr>
<td>Seizure frequency (months)</td>
<td>67.7 (10 - &gt; 100)</td>
<td>17 (5 - 50)</td>
<td>15.3 (2 - 30)</td>
</tr>
<tr>
<td>Extent of resection</td>
<td>GTR (88.9%); PR (11%)</td>
<td>GTR (55%); GTR/MSR (33%); PR (11%)</td>
<td>GTR (66.6%); GTR/MSR (33%)</td>
</tr>
<tr>
<td>HS: present/absent</td>
<td>–</td>
<td>3/7</td>
<td>3/7</td>
</tr>
<tr>
<td>Postoperative epilepsy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engel’s class</td>
<td>I (89%); II (11%)</td>
<td>I (78%); II (22%)</td>
<td>I (89%); II (11%)</td>
</tr>
</tbody>
</table>

CPS, complex partial seizures; SGS, secondary generalized seizures; GTR, gross-total resection; MSR, mesial structures resection; PR, partial resection; HS, hippocampal sclerosis.

All patients underwent presurgical evaluation [144]. In all patients, the lesion was localized by brain MRI; electroencephalographic recordings were performed to detect the epileptogenic area. All lesions were surgically treated and complete removal was accomplished in 25 patients (92.6%). The extent of resection was determined by reviewing the operative report and postoperative MRI investigations. We classified the postoperative seizure outcome according to Engel [145]. Class I consisted of patients who remained completely seizure free and class II includes patients who are almost seizure free or have rare or nocturnal seizures only. Follow-up period ranged from 1 to 15 years.

**Tissue and slices preparation**

Tissue was fixed in 10% buffered formalin (autopsy tissue for two weeks; surgical specimens for 12-24 hours). Two representative paraffin blocks per case (containing the complete lesion or the largest part of the lesion resected at surgery) were sectioned, stained and assessed. Formalin fixed, paraffin-embedded tissue was sectioned at 6 µm and two slices of each paraffin block were used for staining with each antibody (Ab) using adjacent serial sections.
All slices were counterstained with hematoxylin. Two additional slices of each paraffin block were used for Nissl staining. Double-immunostaining experiments were carried out using two slices per paraffin block for co-localization of each inflammatory protein with glial or neuronal markers. Histological and immunocytochemical reactions were done as described below using slices mounted on organosilane-coated slides (SIGMA, St. Louis, MO, USA).

**Antibody characterization and immunocytochemistry**

Glial fibrillary acidic protein (GFAP; polyclonal rabbit, DAKO, Glostrup, Denmark; 1:4000), vimentin (mouse clone V9, DAKO; 1:400), neuronal nuclear protein (NeuN; mouse clone MAB377, IgG1, Chemicon, Temecula, CA, USA; 1:1000), microtubule-associated protein (MAP2; mouse clone HM2, IgG1, SIGMA; 1:100 and polyclonal rabbit, Chemicon; 1:1000), synaptophysin (polyclonal rabbit, DAKO; 1:200), CD34 (QBEnd10; mouse IgG1, Immunotech, Marseille, Cedex, France; 1:600), HLA-DR (mouse clone Tal1b5, SIGMA; 1:100) and CD68 (mouse clone PG-M1, DAKO; 1:200) were used in the routine immunocytochemical analysis of FCD, GG and DNT specimens to document the presence of a heterogeneous population of cells. For the detection of the IL-1β family members the following antibodies (Abs) were used: anti-human IL-1β, goat polyclonal Ab (1:70; sc-1250, Santa Cruz Bio., CA, USA); anti-human IL-1RI, goat polyclonal Ab (1:50; R&D Systems, Abingdon, UK); anti-human IL-1RII, goat polyclonal Ab (1:10; R&D Systems, Abingdon, UK) and anti-human IL-1Ra, goat polyclonal Ab (1:10; R&D Systems, Abingdon, UK). The specificity of these antibodies was tested by pre-incubating the antibodies with a 100-fold excess of the antigenic peptides (Santa Cruz Bio. and R&D Systems). Paraffin-embedded human specimens of prostate carcinoma [251], endometrium [252], gliomas [253, 254] and multiple sclerosis [255] were used as positive controls for immunocytochemical staining.

Immunocytochemistry was carried out as previously described [113, 256]. Both autopsy and surgical specimens were placed into sodium citrate buffer (0.01 M, pH 6.0) and heated in a microwave oven (650 W for 10 minutes). Then sections were incubated for 1 h at room temperature (RT) followed by incubation at 4°C overnight with primary antibodies (IL-1β, IL-1RI, IL-1RII and IL-1Ra). Single-label immunocytochemistry was performed using the avidin-biotin peroxidase method and 3,3’-diaminobenzidine (DAB) as chromogen. Sections were counterstained with hematoxylin. Sections incubated without the primary Ab, with pre-immune sera or with the Ab pre-incubated with the antigenic peptide were essentially blank. For double-labeling studies, sections, after incubation with primary antibodies GFAP, vimentin, Tal1b5, or NeuN, combined with IL-1β, IL-1RI, IL-1RII and IL-1Ra, were incubated for 2 h at RT with Texas Red- or FITC-conjugated anti-goat, anti-mouse or anti-rabbit IgG (1:100; DAKO or Jackson Immuno Research, USA). Sections were then analyzed by means of a laser scanning confocal microscope (Bio-Rad, Hercules, CA, USA; MRC1024) equipped with an argon-ion laser.

**Evaluation of immunostaining and cell counting**

The evaluation of specific immunostaining, the presence or absence of various histopathological parameters and cell counting were performed by two independent observers blind to the identification codes of the samples. The overall concordance was > 90% and the overall kappa value ranged from 0.87 to 0.98. In case of disagreement, independent reevaluation was performed by both observers to define the final score. To minimize staining variability due to procedural parameters (tissue fixation and preservation), we selected 6
cases (see above) in which the lesion and the perilesional zone were represented in the same paraffin block. In GNT cases, the perilesional zone did not include the infiltration zone of the tumor. In GG, to distinguish neoplastic from normal brain tissue we used specific immunocytochemical staining, such as CD34 staining, which is highly expressed in GG and is an helpful diagnostic tool [29, 31].

**Semi-quantitative evaluation of immunoreactivity**

This analysis was done in the different types of pathology using an Olympus Vanox microscope and examining in each slice 100 high-power non-overlapping fields (of 0.0655 mm X 0.0655 mm width; each corresponding to 4.290 µm²) defined in the center of the lesion using a square grid inserted into the eyepiece. A total microscopical area of 858.050 µm² was assessed per case (200 high-power fields). Neuronal cell bodies were differentiated from glia and glioneuronal balloon cells on the basis of morphology. Only neurons in which the nucleolus could be clearly identified were included. Balloon cells have eccentric nuclei and ballooned opalescent eosinophilic cytoplasm. The intensity of IL-1β, IL-1RI, IL-1RII and IL-1Ra staining in each cell type was evaluated using a semi-quantitative scale ranging from 0 to 3 (intensity score; 0: -, no; 1: +/-, weak; 2: +, moderate; 3: ++, strong staining). This score represents the predominant cell staining intensity found in each slice for the different cell types (neurons, astrocytes, microglial cells and balloon cells) as averaged from the selected fields (see above).

**Frequency of cell staining**

In each slice, we assessed the number of neurons, glial or balloon cells labeled by a specific Ab on the total number of each cell type within the lesion using an ocular grid, as previously described [113]. This semi-quantitative frequency score was assigned using 3 distinct categories: (1) < 10%, rare; (2) 11-50% sparse; (3) > 50% high. The product of the intensity and the frequency scores was taken to give the total immunoreactivity score, as previously reported [191, 257].

**Cell counting**

A quantitative approach was used to examine the correlation between the number of cells expressing the inflammatory markers within the lesion and the clinical features of the corresponding patients. The number of positive cell types (neurons, astrocytes, microglia and balloon cells) were quantified as previously described [107, 108]. Three representative digital photos per slice (10 x magnification) were obtained from FCD, GNT and control cortex using an Olympus Vanox microscope equipped with a DP-10 digital camera (Olympus, Japan). Each of the three images spanned a 0.5 mm² region in the center of the lesion and was collected using image acquisition and analysis software (Phase 3 Image System integrated with Image Pro Plus; Media Cybernetics, Silver Spring, MD). The operator outlined all individual cell types within the captured image and an automated cell count was generated. Data obtained in each of the three 0.5 mm² regions per slice were averaged, thus providing a single value for each slice. Values of two slices in each of the two paraffin blocks per case were averaged and this value was used for statistical analysis of data. For statistical analysis of data, SPSS for Windows was used. Data were analyzed with non-parametric Kruskal-Wallis test, followed by a Mann-Whitney test to assess the difference between groups. Correlation between the
number of immunopositive cells and different clinical variables (duration of epilepsy, seizure frequency, age at surgery, age at seizure onset, epilepsy outcome) was assessed using the Spearman’s rank correlation test. The value of p < 0.05 was defined statistically significant.

RESULTS

Case material and histological features
The clinical features of the cases included in this study are summarized in Table 1. All patients had a history of chronic pharmacoresistant epilepsy. Postoperatively, 23 patients (85%) were completely seizure free. In this study, we excluded patients with mild degree of cortical dysplasia and no detectable lesion on MRI, which could represent a non-specific pathological change associated with long-term seizure activity. The FCD cases included in this study had all the previously described histopathological features of severe (type IIB) FCD, including laminar disorganization, neuronal heterotopia and presence of immature neurons, giant neurons, dysmorphic neurons and balloon cells [15]. GGs were composed histologically of a mixture of atypical neuronal cells and neoplastic astrocytes and showed a broad spectrum of histopathological features [29]. The neuronal component, variable in amount, was represented by cells with lack of uniform orientation, abnormal shape and often vesicular nuclei and prominent nucleoli. The glial component of GG consisted mainly of fibrillary astrocytes with different degree of cellularity and strong immunoreactivity for GFAP. DNTs showed a complex nodular or multinodular intracortical architecture with a typical heterogeneous cellular composition. They contained a complex mixture of neuronal cells (often floating in a pale, eosinophilic matrix), astrocytes and a prominent population of oligodendroglia-like cells [258]. Association with cortical dysplasia was found in 4 GNT cases (1 GG and 3 DNT; [259, 260]).

Expression of various members of the IL-1β family in normal human brain and perilesional areas
Neuronal labeling in normal control cortex was not found by immunocytochemistry utilizing specific antibodies against IL-1β, IL-1RI, IL-1RII and IL-1Ra in agreement with previous studies (Fig. 1A, B, G, H; Fig. 3A, B, I, J; Fig. 4) [94, 243, 253, 261, 262]. Resting glial cells in both white and gray matter were also negative for the different antibodies (Fig. 1A, B, G, H; Fig. 3A, B, I, J; Fig. 4). In one autopsy case only, weak IL-1β immunoreactivity (IR) was observed in scattered glial and neuronal cells (Fig. 4A and B). There were no differences between histological normal cortex of surgical specimens (n=6) and control autopsy cortex (n=6), as well as between autopsy (or surgical specimens) at different ages. Perilesional histologically normal cortex (n=6), did not show detectable staining for IL-1β, IL-1RII, IL-1Ra (data not shown) and IL-1RI (Fig. 2L). However, glial and neuronal IL-1β and IL-1RI IR was observed in GNT cases (3 complex form of DNT) with diffuse perilesional reactive gliosis (Fig. 2I and K), and in GNT cases (1 GG and 3 complex form of DNT) in which the tumor was bordered by dysplastic cortex (Fig. 2H). As a note of caution, the absence of IR may only indicate that protein expression is below immunocytochemical detection. Thus, we cannot exclude that these proteins are present, although at low levels, in neurons and/or glia as suggested also by the very low expression of genes coding for IL-1 family members (for review see
The specificity of the immunolabeling in FCD and GNT specimens is supported by the selective intralesional overexpression of IR signals, meaning that no detectable IR for the different IL-1β system proteins was observed in the same specimen within perilesional histologically normal cortex.

**IL-1β system in FCD**

IR for all components of the IL-1β system was encountered in all specimens examined in either neuronal or glial elements. The majority of cases displayed moderate to strong IL-1β (Fig. 1C-F) and IL-1R1 (Fig. 1I-J) IR, whereas weak to moderate IL-1RII (Fig. 3C-E) and IL-1Ra (Fig. 3K-L) IR was observed. Both membranous and cytoplasmic IL-1RI IR was detected in neuronal and glial cells, including both astrocytes and microglia. Noteworthy, both the intensity and the frequency of the neuronal and glial staining of IL-1RII and IL-1Ra were less than those of IL-1β and IL-1RI IR. Fig. 4 shows the immunoreactivity score in the different cell types (neurons, astrocytes, microglial cells and balloon cells). Double-labeling experiments confirmed that IL-1β, IL-1R1, IL-1RII and IL-1Ra were expressed in neurons (NeuN-positive cells; IL-1β: Fig. 1D; IL-1R1: Fig. 1J; IL-1Ra: Fig. 3L; IL-1RII: data not shown), in astrocytes (vimentin-positive cells; IL-1β: Fig. 1C insert b; IL-1Rs and IL-1Ra: data not shown), in microglia (HLA-DR-positive cells; IL-1β: Fig. 1E; IL-1Rs and IL-1Ra: data not shown) and in balloon cells (vimentin-positive cells; IL-1β: Fig. 1F; IL-1RI: Fig. 1I; GFAP-positive cells; IL-1RII: Fig. 3D insert a; IL-1Ra: Fig. 3K).

**Neuronal expression of IL-1β system in FCD**

*IL-1β.* Moderate to strong staining (intensity scores 2-3; arrows in Fig. 1C-E) was observed in > 50% of dysplastic neurons in 7 out of 9 cases; in the remaining 2 cases, moderate IL-1β IR (intensity score 2) was found in 11-50% of dysplastic neurons.

*IL-1RI.* Moderate to strong IL-1RI IR (intensity scores 2-3; Fig. 1J) was detected in 11-50% of neuronal cells in 4 out of 9 cases. In the remaining cases, weak IL-1RI IR (intensity score 1) was found in 11-50% (3 cases) and in < 10% (2 cases) of neuronal cells.

*IL-1RII.* Weak IL-1RII IR (intensity score 1; arrow in Fig. 3E) was detected in < 10% of neuronal cells in 7 out of 9 cases. The remaining cases did not show detectable IL-1RII IR.

*IL-1Ra.* Weak IL-1Ra IR (intensity score 1) was detected in < 10% of neuronal cells in 2 out of 9 cases. Moderate to strong IL-1Ra IR (intensity scores 2-3; arrows in Fig. 3L) was detected in 11-50% (3 cases) and in 10% of neuronal cells (3 cases), whereas strong IL-1Ra IR (intensity score 3) was observed in > 50% of neuronal cells in one case only.

**Glial expression of IL-1β system in FCD**

*Astrocytes*  

*IL-1β.* Strong IL-1β staining (intensity score 3; arrowheads in Fig. 1C and insert a in Fig. 1C) was detected in > 50% of reactive astrocytes in 7 out of 9 cases. In the remaining cases, moderate IR (intensity score 2) was found in > 50% of astrocytes.

*IL-1RI.* Moderate IL-1RI IR (intensity score 2) was detected in 11-50% of reactive astrocytes in 4 out of 9 cases. Weak IL-1RI IR (intensity score 1; arrow in Fig. 1I) was found in > 50% (3 cases) and in < 10% of astrocytes in the remaining 2 cases.

*IL-1RII.* Weak to moderate IL-1RII IR (intensity scores 1-2; insert in Fig. 3E) was detected in < 10% of astroglial cells in 9 out of 9 cases.

*IL-1Ra.* Weak to moderate IL-1Ra IR (intensity scores 1-2) was detected in < 10% of astroglial
cells in 4 out of 9 cases. Moderate IR (intensity score 2) was found in 11-50% of astrocytes in the remaining 5 cases.

**Figure 1. Cell-type distribution of IL-1β and IL-1RI immunoreactivity (IR) in FCD**

Panels A-F: representative photomicrographs of immunocytochemical staining for IL-1β. Panels A-B: IL-1β in control brain. No neuronal or glial labeling is observed in control normal cortex (A) and white matter (B). Panels C-F: IL-1β in FCD. Panel C: strong IL-1β IR is observed in large dysplastic neurons (single arrow), glial cells (single arrowheads) and in balloon cells (double arrowheads) within the dysplastic cortex (FCDIIB). Inserts in panel C show IL-1β IR in a bizarre multinucleated astroglial cell (a), and the co-localization of IL-1β (red) with vimentin (VIM; green) in astroglial cells (b). Panel D: IL-1β IR in dysplastic neurons of different size and morphology (arrows) and in cells with microglial morphology (arrowhead). Insert in panel D: merged image showing co-localization of IL-1β (red) with NeuN (green) in a dysplastic neuron. Sections are counterstained with hematoxylin. Scale bar in A; A-E, G-H: 50 µm; F-I: 30 µm; J: 25 µm.

**Microglia**

IL-1β. Activated microglial cells showed moderate to strong IL-1β staining (intensity scores 2-3; arrowheads in Fig. 1E) in 7 out of 9 cases (> 50% positive microglial cells in 2 cases; 11-50% in 5 cases). In the remaining 2 cases, activated microglial cells (> 50% and 11-50% respectively) showed weak IL-1β staining (intensity score 1).

IL-1RI. Weak to moderate IL-1RI IR (intensity scores 1-2) was detected in 11-50% of activated microglial cells in 5 out of 9 cases. Detectable microglial IL-1RI IR was not found in the remaining 4 cases.
IL-1β system in glioneuronal lesions

Figure 2. Cell-type distribution of IL-1β and IL-1RI immunoreactivity (IR) in GNT Panels A-C: representative photomicrographs of immunocytochemical staining for IL-1β in GG. Panels A-B show strong IL-1β IR in neurons of different size and shape (arrows in panel A), including multinucleated cells (arrow in panel B). IL-1β IR is also observed in the glial component of the tumor (arrowheads in panel A). Insert in panel A: merged image showing co-localization of IL-1β (red) with vimentin (VIM; green) in a tumor astrocyte. Insert in panel B: merged image showing co-localization of IL-1β (red) with NeuN (green). Panel C: IL-1β IR in cells with microglial morphology (arrows) surrounding a neuron (arrowhead). Insert in panel C: merged image showing co-localization of IL-1β (red) with HLA-DR (green) in a perineuronal microglial cell. Panels D-F: IL-1RI in GG. Panel D: strong IL-1RI IR in neurons (arrows) and tumor astrocytes (arrowheads). Panel E: IL-1RI IR in multinucleated astroglial cells (arrows). Insert in panel E: merged image showing co-localization of IL-1RI (red) with vimentin (VIM; green) in a tumor astrocyte. Panel F: IL-1RI IR in a binucleated neuron with membranous and cytoplasmic staining (arrows). Insert in panel F: merged image showing co-localization of IL-1RI (red) with NeuN (green). Panel G: IL-1β in DNT, showing strong IL-1β IR in the neuronal component of the tumor (arrow; floating neuron) and in few cells with astroglial morphology (arrowheads). No notable IR was found in oligodendroglialike cells (insert in panel G). Panels H-I: perilesional dysplastic cortex adjacent to DNT, showing neuronal (H) and astroglial (I) IL-1β immunostaining, as depicted by arrows. Panel J: IL-1RI in DNT, showing strong IL-1RI IR in the neuronal component of the tumor (arrow) and in few cells with astroglial morphology (arrowheads). Panels K-L: perilesional zone adjacent to DNT. Panel K shows IL-1RI IR in perilesional reactive astrocytes. Panel L: histological normal zone without detectable IL-1RI IR. Sections are counterstained with hematoxylin. Scale bar in C; A-C, G, J, K: 40 µm; D: 60 µm; E, H, I: 30 µm; F: 25 µm; L: 65 µm.

IL-1RII. Weak to moderate IL-1RII IR (intensity scores 1-2) was detected in < 10% of activated microglial cells in 6 out of 9 cases. Weak IL-1RII IR (intensity score 1) was detected in > 50% of activated microglial cells in 1 case. Detectable microglial IL-1RII IR was not found in the remaining 2 cases.

IL-1Ra. Weak to moderate IL-1Ra IR (intensity scores 1-2) was detected in < 10% of activated microglial cells in 3 out of 9 cases. Detectable microglia staining was not found in the remaining cases.
Balloon cells

IL-1β. Strong staining (intensity score 3) (double arrowheads in Fig. 1C, and arrow in Fig. 1F) was observed in > 50% of balloon cells in 6 out of 9 cases, whereas in the remaining 3 cases > 50% of balloon cells showed moderate IR (intensity score 2).

IL-1RI. Moderate to strong IL-1RI staining (intensity scores 2-3) was found in 11-50% of balloon cells in 7 out of 9 cases. In the remaining 2 cases < 10% of balloon cells showed weak IL-1RI IR (intensity score 1; asterisk in Fig. 1I).

IL-1RII. 1-10% of balloon cells showed IL-1RII IR in 6 out of 9 cases and 11-50% in the remaining 3 cases. Strong IL1-RII IR (intensity score 3; Fig. 3D) was observed in 2 out of 9 cases, while the remaining cases displayed weak to moderate IR (intensity scores 1-2; Fig. 3C).

IL-1Ra. 11-50% of balloon cells were immunopositive for IL1Ra in 6 out of 9 cases and 1-10% in the remaining 3 cases. Strong IL-1Ra IR (intensity score 3) was observed in 2 out of 9 cases, whereas the remaining cases displayed weak to moderate IR (intensity score 1-2; Fig. 3K).

IL-1β system in GG

IL-1β, IL-1RI and IL1Ra IR was encountered in all specimens examined in either neuronal or glial elements, whereas IL-1RII IR was observed in 89% of the cases. The majority of cases displayed moderate to strong IL-1β (Fig. 2A-C) and IL-1RI (Fig. 2D-F) IR, whereas weak to moderate IL-1RII (Fig. 3F) and IL-1Ra (Fig. 3M) IR was observed. Both membranous and cytoplasmic IL-1RI IR was detected in neuronal and glial cells, including both astrocytes and microglia. Noteworthy, both the intensity and the frequency of the neuronal and glial staining of IL-1RII and IL-1Ra were less than those of IL-1β and IL-1RI IR. Fig. 4 shows the immunoreactivity score in the different cell types (neurons, astrocytes and microglial cells). IL-1β IR was occasionally observed in the endothelial cells of blood vessels (data not shown). Double-labeling experiments confirmed that IL-1β, IL-1RI, IL-1RII and IL-1Ra were expressed in neurons (NeuN-positive cells; IL-1β: Fig. 2B; IL-1RI: Fig. 2F; IL-1RII: Fig. 3F insert b; IL-1Ra: data not shown), in astrocytes (vimentin-positive cells; IL-1β: Fig. 2A; IL-1RI: Fig. 2E; IL-1RII and IL-1Ra: data not shown) and in microglia (HLA-DR-positive cells; IL-1β: Fig. 2C; IL-1Rs and IL-1Ra: data not shown).

Neuronal expression of IL-1β system in GG

IL-1β. Strong IL-1β IR (intensity score 3) (arrows in Fig. 2A, B) was detected in > 50% of neuronal components in 8 out of 9 cases. In the remaining case, moderate IL-1β IR (intensity score 2) was found in > 50% of neurons.

IL-1RI. Moderate to strong IL-1RI IR (intensity scores 2-3; arrows in Fig. 2D, F) was detected in neuronal cells in 8 out of 9 cases (> 50% of neurons in 2 cases; 11-50% of neurons in 6 cases). In the remaining case, weak IL-1RI IR (intensity score 1) was observed in 11-50% of neurons.

IL-1RII. Weak to moderate IL-1RII IR (intensity scores 1-2; Fig. 3F insert a) was detected in < 10% of neuronal cells in 5 out of 9 cases. Detectable neuronal IL-1RII IR was not found in 3 out of 9 cases, whereas the remaining case displayed moderate IR (score 2) in 11-50% of neurons.

IL-1Ra. Weak IL-1Ra IR (intensity score 1; arrows in Fig. 3M and insert a) was observed in 2 out of 9 cases (< 10% and 11-50% of neuronal cells respectively). Moderate to strong IL-1Ra IR (intensity scores 2-3) was observed in 11-50% of neuronal cells in 6 out of 9 cases. Strong IL-1Ra IR was observed in > 50% of neuronal cells in the remaining case.
**Glial expression of IL-1β system in GG**

**Astrocytes**

*IL-1β.* Strong IL-1β staining (intensity score 3; arrowheads in Fig. 2A) was detected in > 50% of reactive astrocytes in 8 out of 9 cases. In the remaining case, moderate IR (intensity score 2) was found in > 50% of astrocytes.

*IL-1RI.* Moderate to strong IL-1RI IR (intensity scores 2-3; arrowheads in Fig. 2D and arrows in Fig. 2E) was detected in tumor astrocytes in 6 out of 9 cases (> 50% positive astroglial cells in 3 cases; 11-50% in 3 cases). In the remaining 3 cases, weak IL-1RI IR (intensity score 1) was found in 11-50% of astrocytes.
**IL-1RII.** Weak to moderate IL-1RII IR (intensity scores 1-2; Fig. 3F insert c) was detected in < 10% of astroglial cells in 7 out of 9 cases. Detectable astroglial IL-1RII IR was not found in 1 case, whereas the remaining case displayed moderate IR (intensity score 2) in 11-50% of tumor astrocytes.

**IL-1Ra.** Weak to moderate IL-1Ra IR (intensity scores 1-2; Fig. 3M insert b) was detected in < 10% of astroglial cells in 2 out of 9 cases. Moderate IR (intensity score 2) was found in 11-50% of astrocytes in 4 out of 9 cases. Moderate to strong IR (intensity scores 2-3) was detected in > 50% of tumor astrocytes in the 3 remaining cases.

**Microglia**

**IL-1β.** Activated microglial cells showed moderate to strong IL-1β staining (intensity scores 2-3; arrows in Fig. 2C) in 8 out of 9 cases (> 50% positive microglial cells in 2 cases; 11-50% in 6 cases). In the remaining case, activated microglial cells (11-50%) showed weak IL-1β staining (intensity score 1).

**IL-1RI.** Moderate IL-1RI IR (intensity score 2) was detected in 11-50% of activated microglial cells in 2 out of 9 cases. Weak IL-1RI IR (intensity score 1) was detected in 5 out of 9 cases in > 50% in 1 case and < 10% of activated microglial cells in 4 cases out of 9 cases, respectively. Detectable IL-1RI IR was not found in microglia in the remaining 2 cases.

**IL-1RII.** Weak to moderate IL-1RII IR (intensity scores 1-2; Fig. 3F insert d) was detected in < 10% of activated microglial cells in 3 out of 9 cases. Detectable microglial IL-1RII IR was not found in 5 cases. Weak IL-1RII IR (intensity score 1) was detected in > 50% of activated microglial cells in the remaining case.

**IL-1Ra.** Weak to moderate IL-1Ra IR (intensity scores 1-2; Fig. 3M insert c) was detected in < 10% of activated microglial cells in 7 out of 9 cases. Weak to moderate IL-1Ra IR (intensity scores 1-2) was detected in > 50% of activated microglial cells in the remaining 2 cases.

**IL-1β system in DNT**

IL-1β, IL-1RI and IL1Ra IR was encountered in all specimens examined in either neuronal or glial elements, whereas IL-1RII IR was observed in 89% of the cases. The majority of cases displayed moderate to strong IL-1β (Fig. 2G) and IL-1RI (Fig. 2J) IR, whereas weak to moderate IL-1RII (Fig. 3G) and IL-1Ra (Fig. 3N) IR was observed. Both membranous and cytoplasmic IL-1RI IR was detected in neuronal and glial cells, including both astrocytes and microglia. Noteworthy, both the intensity and the frequency of the neuronal and glial staining of IL-1RII and IL-1Ra were less than those of IL-1β and IL-1RI IR. Fig. 4 shows the immunoreactivity score in the different cell types (neurons, astrocytes and microglial cells). IL-1β IR was occasionally observed in the endothelial cells of blood vessels (data not shown). Double-labeling experiments confirmed that IL-1β, IL-1RI, IL-1RII and IL-1Ra were expressed in neurons (data not shown), in astrocytes (data not shown) and in microglia (data not shown).

**Neuronal expression of IL-1β system in DNT**

**IL-1β.** Strong IL-1β IR (intensity score 3) (arrow in Fig. 2G) was detected in > 50% of neuronal components in 7 out of 9 cases. In the remaining 2 cases moderate IL-1β IR (intensity score 2) was found in > 50% of neurons.

**IL-1RI.** Moderate to strong IL-1RI IR (intensity scores 2-3; arrow in Fig. 2J) was detected in neuronal cells in 8 out of 9 cases (> 50% positive neuronal cells in 4 cases; 11-50% in 4
cases). In the remaining case, weak IL-1RI IR (intensity score 1) was observed in < 10% of neurons.

**IL-1RII.** Weak to moderate IL-1RII IR (intensity scores 1-2; arrow in Fig. 3G) was detected in < 10% of neuronal cells in 8 out of 9 cases. Detectable IL-1RII IR was not found in the remaining case.

**IL-1Ra.** Weak IL-1Ra IR (intensity score 1; arrow in Fig. 3N) was observed in < 10% of neuronal cells in 5 out of 9 cases. Moderate to strong IL-1Ra IR (intensity scores 2-3) was observed in < 10% of neuronal cells in 3 out of 9 cases and in 11-50% in the remaining case.

**Glial expression of IL-1β system in DNT**

**Astrocytes**

**IL-1β.** Strong IL-1β staining (intensity score 3; arrowheads in Fig. 2G) was detected in > 50% of reactive astrocytes in 6 out of 9 cases. In the remaining 3 cases, moderate IR (intensity score 2) was found in 11-50% of astrocytes.

**IL-1RI.** Moderate to strong IL-1RI IR (intensity scores 2-3; arrowheads in Fig. 2J) was detected in 11 to > 50% of reactive astrocytes in 4 out of 9 cases (> 50% positive astroglial cells in 3 cases; 11-50% in 1 case). In the remaining cases, weak IL-1RI IR (intensity score 1) was found in > 50% (1 case), 11-50% (1 case) and < 10% (3 cases) of astrocytes.

**IL-1RII.** Weak to moderate IL-1RII IR (intensity scores 1-2) was detected in < 10% of astroglial cells in 5 out of 9 cases. Detectable astroglial IL-1RII IR was not found in the remaining 4 cases.

**IL-1Ra.** Weak IL-1Ra IR (intensity score 1) was detected in < 10% of astroglial cells in 3 out of 9 cases. Moderate IR (intensity score 2) was found in 11-50% of astrocytes in 1 case, whereas the remaining 5 cases did not show detectable astroglial staining.

**Microglia**

**IL-1β.** Activated microglial cells showed moderate to strong IL-1β staining (intensity scores 2-3) in 8 out of 9 cases (> 50% positive microglial cells in 5 cases; 11-50% in 3 cases). In the remaining case, activated microglial cells (< 10%) showed weak IL-1β staining (intensity score 1).

**IL-1RI.** Weak to moderate IL-1RI IR (intensity scores 1-2) was detected in < 10% (3 cases) and in 11-50% (2 cases) of activated microglial cells. Strong IL-1RI IR (intensity score 3) was found in > 50% (1 case) and < 10% (1 case) of activated microglial cells. Detectable microglial IL-1RI IR was not found in the remaining 2 cases.

**IL-1RII.** Weak IL-1RII IR (intensity score 1) was detected in < 10% of activated microglial cells in 1 out of 9 cases. Detectable microglial IL-1RII IR was not found in the remaining 8 cases.

**IL-1Ra.** Weak IL-1Ra IR (intensity score 1) was detected in < 10% of activated microglial cells in 3 out of 9 cases. Detectable microglia staining was not found in the remaining 6 cases.

**Oligodendroglia**

Detectable IR for the different IL-1β family members analyzed was not observed in the oligodendroglial component of DNT (IL-1β: insert in Fig. 2G; IL-1RI: Fig. 2J; IL-1RII: Fig. 3G; IL-1Ra: Fig. 3N).
Molecular alterations in epilepsy-associated malformations of cortical development

IL-1β family members and clinical features of FCD and GNT

We examined the correlation between the number of IL-1β, IL-1RI, IL-1RII and IL-1Ra immunopositive neuronal and astroglial cells within the different types of lesion and various clinical variables, such as age at surgery, age at seizure onset, duration of epilepsy, preoperative seizure frequency and seizure outcome after surgery. The percentage of IL-1β, IL-1RI, IL-1RII and IL-1Ra immunopositive neuronal and astroglial cells in FCD, GG and DNT specimens is reported in Table 2. The number of IL-1β- and IL-1RI-positive neuronal cells in FCD, GG and DNT, was positively correlated with the frequency of seizures prior to surgical resection (Fig 5A-C; Spearman rank correlation coefficient: IL-1β: FCD, r = 0.740, p < 0.05; GG, r = 0.966, p < 0.01; DNT, r = 0.815, p < 0.01; IL-1RI: FCD, r = 0.840, p < 0.01; GG, r = 0.899, p < 0.01; DNT, r = 0.837, p < 0.01). Significant correlation was not found between the number of IL-1β- and IL-1RI-positive astrocytes and the frequency of seizures or between the number of IL-1β- and IL-1RI-positive astrocytes and neuronal cells and the duration of epilepsy (data not shown). The number of IL-1Ra-positive neurons and astrocytes was negatively correlated with the duration of epilepsy in both FCD and GG cases (Fig 5D-E;
Spearman rank correlation coefficient: IL-1Ra FCD: astrocytes r = -0.912, p < 0.01; neurons r = -0.837, p < 0.01; IL-1Ra GG: astrocytes r = -0.867, p < 0.01; neurons r = -0.733, p < 0.05).

For the DNT specimens, there was a tendency for the IL-1Ra IR to be negatively correlated to the duration of epilepsy (IL-1Ra astrocytes r = -0.340, p > 0.05; neurons r = -0.293; p > 0.05).

The number of IL-1β-, IL-1RI-, IL-1RII- and IL-1Ra-positive microglial and balloon cells did not correlate with the frequency of seizures or the duration of epilepsy. Significant correlation was not found between the number of IL-1β, IL-1RI, IL-1RII and IL-1Ra neuronal and glial positive cells and other clinical variables such as age at surgery, age at seizure onset or seizure outcome after surgery.

Table 2. Percentage of IL-1β, IL-1RI, IL-1RII and IL-1Ra immunopositive neuronal and astroglial cells in FCD, GG and DNT specimens

<table>
<thead>
<tr>
<th>Antibody</th>
<th>FCD (n=9)</th>
<th>GG (n=9)</th>
<th>DNT (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neurons</td>
<td>Astrocytes</td>
<td>Neurons</td>
</tr>
<tr>
<td>IL-1β</td>
<td>63 ± 9.7%</td>
<td>76 ± 9%</td>
<td>66 ± 4.5%</td>
</tr>
<tr>
<td>IL-1RI</td>
<td>33 ± 13%</td>
<td>53 ± 7%</td>
<td>34 ± 8%</td>
</tr>
<tr>
<td>IL-1RII</td>
<td>9 ± 0.6%</td>
<td>12 ± 2%</td>
<td>11 ± 0.5%</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>28 ± 9%</td>
<td>23.2 ± 6%</td>
<td>23.6 ± 5%</td>
</tr>
</tbody>
</table>

Focal cortical dysplasia (FCD), ganglioglioma (GG) and dysembryoplastic neuroepithelial tumor (DNT). Data are expressed as mean ± SEM.

Figure 5. Correlation between the number of IL-1β-, IL-1RI- and IL-1Ra-positive cells and clinical variables

Panels A-C: Scatter plots showing the significant positive correlation between the density of IL-1β- and IL-1RI-immunopositive neurons and seizure frequency (seizures per month) in FCD (A), GG (B) and DNT (C). Spearman rank correlation coefficient: IL-1β: FCD, r = 0.740, p < 0.05; GG, r = 0.966, p < 0.01; DNT, r = 0.815, p < 0.01; IL-1RI: FCD, r = 0.840, p < 0.01; GG, r = 0.899, p < 0.01; DNT, r = 0.837, p < 0.01. Panels D-E: Scatter plots showing the significant negative correlation between the density of IL-1Ra immunoreactive neuronal and astroglial cells and the duration of epilepsy (years) in FCD (D) and GG (E). Spearman rank correlation coefficient: IL-1Ra: FCD: astrocytes r = -0.912, p < 0.01; neurons r = -0.837, p < 0.01; GG: astrocytes r = -0.867, p < 0.01; neurons r = -0.733, p < 0.05.
DISCUSSION

Focal cortical dysplasia and glioneuronal tumors are major causes of medically intractable epilepsy in young patients; however, the cellular mechanisms underlying the intrinsic and high epileptogenicity of these lesions remain to be fully investigated. It has been postulated that IL-1β-mediated signaling is involved in the pathogenesis of seizures, possibly playing a critical role in epileptogenesis (reviewed in [82, 241]). In the present study, we show that IL-1β, IL-1Ra and IL-1β receptors are expressed in lesional tissue of FCD and GNT specimens, but not in histologically normal control tissue. The number and type of cells expressing IL-1β, IL-1RI and IL-1Ra correlate with the patient’s clinical course. The cell-specific distribution of these molecules in relation with the epileptogenicity of these developmental lesions is discussed below.

**IL-1β family members in neuronal cells of FCD and GNT specimens**

Recent findings indicate that neuronal cells represent a source of brain IL-1β in pathological conditions including epilepsy [94, 255, 264, 265]. In our study, both IL-1β and IL-1RI proteins were detected in the neuronal component of all FCD and GNT specimens, including dysmorphic neurons in FCD and large atypical neurons in GNT. The strong neuronal expression of IL-1β and IL-1RI proteins may be intrinsic to these developmental lesions or induced by seizures, or both. A clue for the interpretation of these findings is provided by studies in experimental models. In particular, IL-1β IR was described in surviving pyramidal and hilar neurons in the sclerotic hippocampus of chronic epileptic rats. Moreover, neuronal expression of IL-1RI is induced by status epilepticus in the hippocampus where ongoing cell degeneration occurs [94, 266]. It is likely therefore that the persistent neuronal up-regulation of IL-1β and its signaling receptor in human epileptic tissue is intrinsic to the developmental lesion per se or is related to the concomitant occurrence of seizures and the neuropathology. Seizures alone are unlikely to be responsible for the observed effects because perilesional tissue with normal morphology, but exposed to seizures, did not express IL-1β or its receptor. The autocrine or paracrine activation of neuronal IL-1RI by IL-1β released by neurons (and/or glial cells, see later) may contribute to the recurrence of seizure activity in these patients. Thus, experimental evidence indicates that an increase in brain IL-1β results in proconvulsant effects [93, 94, 96, 97]. Accordingly, the density of IL-1β and IL-1RI-positive neurons in human specimens is positively correlated with the frequency of seizures prior to surgery.

An important observation is that the aberrant neurons expressing IL-1β and its signaling receptor do not express markers of apoptosis [108, 109], although this cytokine was shown to promote neuronal cell death in pathological conditions such as ischemia/hypoglycemia, stroke and excitotoxicity [83, 267]. This phenomenon should be considered in light of the neurotrophic effects of IL-1β, which depend both on its concentration and the length of time the tissue is exposed to this cytokine [267-269]. These neurotrophic effects are likely to be mediated via the production of nerve growth factors [270, 271] and different types of neurotrophin receptors are highly expressed within the neuronal components of both FCD and GNT specimens [240, 256]. Thus, the IL-1β system could influence, early during development, the fate and survival of the abnormal (hyperexcitable) neurons in these developmental lesions. These neurons may then provide a persistent source of cytokine in the affected tissue, thus contributing to decrease the excitability threshold.
Although IL-1β function is directly mediated by the IL-1RI, two members of this system, IL-1RII and IL-1Ra, play a crucial role in modulating the IL-1β signaling pathway. IL-1RII, lacking the intracellular receptor domain, acts as ‘decoy’ receptor by binding IL-1β and preventing its interaction with IL-1RI [272, 273]. IL-1RII IR in the dysplastic cortex of FCD and in GNT specimens was detected in ~10% of the neurons. No correlation was found with clinical variables, such as the frequency or the duration of seizures. IL-1RII mRNA is induced by kainate seizures in rat neurons only transiently and is not detected in brain of chronically epileptic rats [84].

We observed an enhanced neuronal expression of IL-1Ra, a competitive antagonist of IL-1β at IL-1RI [274] in FCD and GNT specimens; however, both the intensity of neuronal staining and the number of positively-stained neurons were less than detected for IL-1β. Accordingly, studies in rodents showed that IL-1Ra is induced transiently in neurons by seizures and brain damage and to a lower extent than IL-1β [83, 94]. The effects of IL-1β are rapidly inhibited by relatively high amounts of IL-1Ra which is typically produced 100-1000 times in excess to IL-1β during peripheral inflammatory reactions [274]. Thus, the relatively scarce expression of IL-1Ra and IL-1RII, as compared to IL-1β and IL-1RI, in neurons in the lesional areas suggests that pivotal mechanisms apt to rapidly terminate the actions of IL-1β are defective in these brain specimens. Because of the transient characteristics of IL-1RII and IL-1Ra induction in experimental models of seizures and brain damage, it is possible that the time period between the last seizure and the tissue preparation had influenced the level of expression of these proteins. These findings indicate that efficient mechanisms to control IL-1β signaling in lesional tissue are lacking and this may contribute to epileptogenicity. In this context, it is interesting to note that the number of IL-1Ra positive neurons was negatively correlated with the duration of epilepsy in these patients.

**IL-1β family members in glial cells of FCD and GNT specimens**

Glial cells represent the main source of brain IL-1β production in several pathological conditions including epilepsy (reviewed in [82-84, 275]). Both activated astrocytes and microglia are highly represented within developmental lesions associated with epilepsy [107-109]. In the present study, we show that both reactive astrocytes in FCD and tumor astrocytes in GNT, as well as microglial cells, express high levels of IL-1β and IL-1RI. Accordingly, high expression of IL-1β was previously detected in glial tumors, such as glioblastoma, anaplastic and pilocytic astrocytomas [253, 254]. The presence of both IL-1β and IL-1RI in glial cells, similarly to what observed in neurons, suggests that IL-1β may subserve both autocrine and paracrine-like actions in lesional tissue. In contrast, IL-1RII and IL-1Ra IR in the dysplastic cortex of FCD and in GNT specimens was detected in less than 30% of the glial cells. Interestingly, the number of IL-1Ra positive astrocytes, as observed also in neurons, was negatively correlated with the duration of epilepsy. As discussed previously for the neuronal expression of this cytokine and its receptors, IL-1β expression in glia in these developmental lesions is not associated with neurotoxic effects. The astroglial expression of IL-1β in FCD and GNT specimens suggests that this cytokine could regulate the proliferation of both reactive and tumor astrocytes [276, 277] by autocrine or paracrine loops. Microglial and astrogial production of IL-1β may be involved also in enhancing neuronal excitability via cytokine-mediated inhibition of glutamate re-uptake by astrocytes leading to increased extracellular glutamate concentrations [278]. Neuronal production of IL-1β can contribute to this effect. In addition, IL-1β has been shown to synergize with the metabotropic glutamate receptor
subtype 3 in the induction of IL-6 release by activated glial cells [279, 280]. This interaction may contribute to the complexity of the chronic inflammatory state in these epileptogenic brain lesions.

Although a rapid IL-1β and IL-1RI expression is induced by seizures in microglia and astrocytes in experimental models [93, 94, 96, 243, 266], seizures alone cannot account for changes in glial expression in these developmental lesions because perilesional tissue was exposed to seizures but devoid of detectable immunostaining. Therefore, as previously discussed for neurons, the lesion per se or the concomitant presence of the lesion and the epileptic activity, are likely to play a role in establishing a chronic inflammatory state in these neuropathologies.

IL-1β family members in balloon cells

Much attention has been focused on giant balloon cells for their role in the epileptogenicity of lesions, such as severe FCD (type IIB) [72, 281, 282]. Whether these cells are glial or neuronal is controversial [205, 283, 284]. We report that balloon cells express IL-1β, suggesting that they represent an additional source of persistent IL-1β production within the dysplastic cortex. They appear to be also a target of this cytokine because they express IL-1RI. IL-1RⅡ and IL-1Ra are also expressed in balloon cells although to a lower extent, as previously observed in all other cellular components. Balloon cells containing mGluRs [113] may also be involved in the functional interactions between IL-1β and the metabotropic glutamatergic system [280, 285].

IL-1β and seizures: mechanisms of action

One crucial mechanism by which IL-1β can increase neuronal excitability likely consists of its ability to enhance glutamate-mediated actions on neurons. This effect can be accomplished at least by two routes; 1: enhancement of extracellular glutamate concentrations; 2: increased function of the N-methyl-D-asparte (NMDA) receptors. In particular, IL-1β has been shown to inhibit glutamate re-uptake in cultures of rat and human astrocytes [278, 286]. Additionally, IL-1β activates inducible nitric oxide (NO) synthase, leading to production of NO and subsequent increase in glutamate release [287, 288]. Molecular and functional interactions between IL-1β and NMDA receptors have been recently described. First, the proconvulsant activity of IL-1β in rodents is blocked by a selective competitive antagonist of NMDA receptors [93]. Second, IL-1β acting on IL-1RI, enhances NMDA receptor function in hippocampal neurons via phosphorylation of the NR2B subunit which regulates calcium influx [87]. This effect of IL-1β was associated with an exacerbation of NMDA-induced increase in intracellular calcium and increased NMDA-dependent neuronal cell loss. Interestingly, both dysplastic neurons and neuronal cells in GNT specimens contain high levels of NMDAR1 and NMDAR2A/B subunit proteins [115, 289, 290], and NMDAR2A/B distribution correlates with in situ epileptogenicity in patients with FCD [291, 292]. In addition to the permissive role on glutamatergic transmission, IL-1β has been reported also to inhibit GABA-mediated Cl⁻ fluxes possibly reducing inhibitory transmission [88, 293].
CONCLUDING REMARKS

GliaL overexpression of different proinflammatory cytokines, including IL-1β, has also been described in tissue of non-epileptic patients with multiple sclerosis (MS) or with neurodegenerative disorders, such as spinocerebellar ataxia type 3 (SCA3), Parkinson’s disease (PD) and Alzheimer’s disease (AD) [255, 261, 294-296]. In these pathologies the activation of the inflammatory pathways appears to be especially involved in the development of neuronal degeneration [296]. The majority of these patients, despite the activation of the IL-1β system in CNS, do not develop seizures. The demyelinating lesions, however, are mainly localized in the deep (periventricular) white matter. Interestingly, in MS patients who developed seizures, a causal relationship has been suggested between the extent of cortical inflammation and the occurrence of epilepsy [297]. As for neurodegenerative disorders, they represent an increasingly recognized cause of epilepsy. Ten to 22% of AD patients and up to 84% of demented individuals with Down Syndrome develop seizures [298, 299]. Recent studies suggest that seizure activity in these patients is the result of the damage of selective cortical circuits. In particular, the degeneration of septal neurons may contribute to the hyperexcitability observed in AD patients [300]. Thus, the role played by IL-1β in distinct neuropathologies and the final outcome of brain inflammation likely depends on the specific brain areas and neuronal circuitry involved.

In summary, our clinical findings demonstrate that chronic inflammation, as exemplified by measuring various members of the IL-1β family in lesional and perilesional areas, indeed occurs in human epileptic tissue from FCD and GNT. Because IL-1β is a cytokine endowed of proconvulsant and ictogenic properties, the chronic expression of IL-1β signaling in aberrant neurons, glia and glio-neuronal entities, such as balloon cells, may contribute to increased network excitability in these developmental lesions. In particular, our data indicate that chronic inflammatory reactions are intrinsic to lesional tissue and sustained by aberrant cells. The recurrence of seizures in lesional tissue likely contributes to perpetuate inflammation. It is worth noting that IL-1Ra and the decoy receptor IL-1RII are expressed within the lesions to a lower extent than IL-1β and its signaling receptor, thus implying that the actions of IL-1β in brain are not under an effective inhibitory control, differently from what happens during inflammatory reactions in the periphery [274]. The up-regulation of IL-1β signaling in these neuropathologies related to epilepsy has important mechanistic and therapeutic implications because it highlights potential new mechanisms of epileptogenesis and seizure recurrence and permits to identify novel putative targets for anticonvulsant and antiepileptogenic intervention.

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