IgG and Complement Deposition and Neuronal Loss in Cats and Humans With Epilepsy and Voltage-Gated Potassium Channel Complex Antibodies

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Abstract
Voltage-gated potassium channel complex (VGKC-complex) antibody (Ab) encephalitis is a well-recognized form of limbic encephalitis in humans, usually occurring in the absence of an underlying tumor. The patients have a subacute onset of seizures, magnetic resonance imaging findings suggestive of hippocampal inflammation, and high serum titers of Abs against proteins of the VGKC-complex, particularly leucine-rich, glioma-inactivated 1 (LG11). Most patients are diagnosed promptly and recover substantially with immunotherapies; consequently, neuropathological data are limited. We have recently shown that feline complex partial cluster seizures with orofacial involvement (FEPSO) in cats can also be associated with Abs against VGKC-complexes/LGI1. Here we examined the brains of cats with FEPSO and compared the neuropathological findings with those in a human with VGKC-complex-Ab limbic encephalitis. Similar to humans, cats with VGKC-complex-Ab and FEPSO have hippocampal lesions with only moderate T-cell infiltrates but with marked IgG infiltration and complement C9neo deposition on hippocampal neurons, as well as other less frequent targets (4, 5), can be both paraneoplastic and nonparaneoplastic (4). The patients often respond well to treatments that reduce the Ab levels (5–7), suggesting that the Abs are pathogenic (8, 9). However, despite positive responses to treatment, the outcomes of encephalitis with Abs to the VGKC complex are not always optimal and cognitive deficits may remain (10).

INTRODUCTION
Antibody (Ab)-associated encephalitides have gained much interest in the last few years (1–3). The Abs can be directed against neuronal intracellular or surface antigens. The “classical” encephalitides with Abs against intracellular or intranuclear antigens, such as Hu, Ma, or Yo, are associated with tumors, and the Abs are not thought to be pathogenic; by contrast, diseases with Abs against surface antigens such as the voltage-gated potassium channel complex (VGKC-complex) or the N-methyl-D-aspartate receptor (NMDAR), as well as other less frequent targets (4, 5), can be both paraneoplastic and nonparaneoplastic (4). The patients often respond well to treatments that reduce the Ab levels (5–7), suggesting that the Abs are pathogenic (8, 9). However, despite positive responses to treatment, the outcomes of encephalitis with Abs to the VGKC complex are not always optimal and cognitive deficits may remain (10).

In recent years, it has been shown that VGKC-complex-Ab- and FEPSO have hippocampal lesions with only moderate T-cell infiltrates but with marked IgG infiltration and complement C9neo deposition on hippocampal neurons, associated with neuronal loss. These findings provide further evidence that FEPSO is a feline form of VGKC-complex-Ab limbic encephalitis and provide a model for increasing understanding of the human disease.

Key Words: Cat, Epilepsy, Feline complex partial cluster seizures with orofacial involvement, hippocampal necrosis, Limbic encephalitis, Voltage-gated potassium channel complex.

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TABLE. Summary of Results in the Hippocampal Region of Cats With Feline Complex Partial Cluster Seizures With Orofacial Involvement, Seizures and Hippocampal Sclerosis, Encephalitic and Normal Feline Controls, and a Human Case of Voltage-Gated Potassium Channel Antibody-Associated Encephalitis

| Animal No. | Age (y) | Sex (f/m) | Breed | Diagnosis | Therapy | MRT | Disease Duration | MRT | VGKC (pmol/L) | LGI1 (Cells/mm²) | CD3 (Cells/mm²) | C9neo Pattern | TUNEL (Cells/mm²) | Neuronal Loss |
|------------|---------|-----------|-------|-----------|---------|-----|------------------|-----|--------------|-----------------|----------------|--------------|----------------|---------------|-------------|
| 1          | 10      | f         | ESH   | FEPSO     | Anti-EP + cort | 5 d | ND               | 123/– | 16.7         | 7.0             | 1             | 8.3           | 4              |               |
| 2          | 7       | f         | ESH   | FEPSO     | Anti-EP + cort | 30 d | HN              | 443/+ | 0.4          | 3.4            | 1             | 13.8          | 5              |               |
| 3          | 7       | m         | ESH   | FEPSO     | Anti-EP + cort | 89 d | Normal          | 791/+ | 0.1           | 0.0            | 0             | 0.0           | 0              |               |
| 4          | ad      | f         | ESH   | FEPSO     | No tx          | 2 d  | ND              | 24/ND | 2.4           | 0.4            | 1             | 3.0           | 3              |               |
| 5          | 2       | f         | ESH   | FEPSO     | Anti-EP          | 8 d  | ND              | ND    | 4.4           | 2.2            | 1             | 15.7          | 4              |               |
| 6          | 2       | f         | ESH   | FEPSO     | Anti-EP          | 10 d | ND              | ND    | 5.5           | 2.3            | 1             | 6.3           | 4              |               |
| 7          | 3       | m         | Siam  | FEPSO     | Anti-EP          | 3 d  | ND              | ND    | 0.9           | 0.2            | 1             | 16.8          | 4              |               |
| 8          | 1       | m         | ESH   | FEPSO     | Anti-EP          | 49 d | ND              | ND    | 4.2           | 0.3            | 1             | 10.6          | 5              |               |
| 9          | 14      | f         | ESH   | FEPSO     | Anti-EP          | 91 d | ND              | ND    | 1.1           | 15.6           | 1             | 29.5          | 5              |               |
| 10         | 2       | m         | ESH   | FEPSO     | Anti-EP          | 2 y 10 m | ND | ND | 0.5 | 38.0 | 1 | 22.8 | 5 |
| 11         | 6       | m         | ESH   | FEPSO     | No tx          | 4 m  | ND              | ND    | 2.1           | 0.0            | 0             | 0.3           | 4              |               |
| 12         | 11      | f         | ESH   | FEPSO     | No tx          | Unknown | ND | ND | 0.1 | 47.2 | 1 | 15.1 | 4 |
| 13         | 1       | f         | ESH   | FEPSO     | Anti-EP          | 6 d  | ND              | ND    | 1.2           | 3.4            | 1             | 2.9           | 5              |               |
| 14         | 7       | m         | ESH   | FEPSO     | No tx          | 3 d  | ND              | ND    | 0.9           | 2.1            | 1             | 4.2           | 4              |               |
| 15         | 12.8    | f         | ESH   | FEPSO     | Anti-EP + cort     | 90 d | HN              | 686/+ | 2.9 | 6.9 | 1 | 0.0 | 4 |
| Average    |         |           |       |           |               |      |                 |       | 2.9 ± 1.1* | 8.6 ± 3.7† | 10.0 ± 2.3‡ |
| Epileptic controls | | | | | | | | | | | | | | | |
| 16         | 3       | f         | ESH   | Seizures/HS | ND | 7 d | ND | ND | 89.4 | 0.0 | 2 | 0.4 | 4 |
| 17         | 8       | f         | Car   | Seizures/HS | ND | 3 d | ND | ND | 44.8 | 0.0 | 3 | 10.0 | 3 |
| 18         | 1       | m         | ESH   | Seizures/HS | ND | 23 d | ND | ND | 3.3 | 0.0 | 0 | 0.0 | 3 |
| Average    | 46 ± 25 | 0.0 ± 0.0 | | | | | | 3.5 ± 3.3   |
| Encephalitic controls | | | | | | | | | | | | | | | |
| 19         | 0.4     | f         | Main  | FIP       | ND | 4 d | ND | ND | 0.4 | 0.0 | 0 | 0.0 | 0 |
| 20         | 1       | f         | ESH   | FIP       | Unknown | ND | ND | 5.6 | 0.0 | 0 | 0.0 | 0 |
| 21         | 10      | f         | ESH   | TOX       | ND | 10 d | ND | ND | 4.9 | 0.0 | 0 | 0.0 | 0 |
| 22         | 3       | m         | ELH   | TOX       | ND | 7 d | ND | ND | 1.3 | 0.0 | 0 | 0.0 | 0 |
| 23         | 4.3     | f         | ESH   | TOX       | ND | 33 d | ND | ND | 0.2 | 0.0 | 0 | 0.0 | 0 |
| Average    | 2.4 ± 1.2 | 0.0 ± 0.0 | | | | | | 0.0 ± 0.0   |
| Normal feline controls | | | | | | | | | | | | | | | |
| 24         | ad      | f         | Ben   | CON       | ND | 0 d | ND | ND | 0.0 | 0.0 | 0 | 0.0 | 0 |
| 25         | 0.6     | m         | BSH   | CON       | ND | 0 d | ND | ND | 0.0 | 0.0 | 0 | 0.0 | 0 |
| Average    | 0.0 ± 0.0 | 0.0 ± 0.0 | | | | | | 0.0 ± 0.0   |
| Human VGKC encephalitis as described in Bien et al (3) | | | | | | | | | | | | | | | |
| VGKC/3     | 59      | m         | NA    | VGKC encephalitis | Unknown | 5 m | ND | 958/ND | 13.2 | 4.4 | 1 | 2.0 | 5 |

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Whereas LE and the ensuing hippocampal atrophy (i.e., hippocampal sclerosis) in humans has been noted for some time, the association between hippocampal necrosis (HN) and temporal lobe epilepsy in cats has been shown in a relatively low numbers of cases (16–20). However, a subgroup of cats with temporal lobe epilepsy displayed feline complex partial seizures with orofacial involvement (FEPSO) (21, 22). Our recent investigations revealed that many of these animals, in addition to facial involvement, have VGKC-complex/LGI1 Abs (23). Therefore, FEPSO in cats has much in common with human VGKC-complex-Ab encephalitis including facial-brochial dystonic seizures (14).

The neuronal damage in VGKC-associated encephalitis cases seems to be caused, at least partly, by complement-mediated mechanisms. In a comparison of 4 VGKC-associated encephalitis patients with other forms of encephalitis, there were low to moderate T-cell infiltrates but gliaosis and activated microglia in the hippocampi with neuronal loss. Strikingly, in hippocampal areas with acute neuronal cell death, there was also evidence of immunoglobulin on the surface of neurons and deposition of complement C9neo, indicating functional complement activation (3).

The aim of the present study was to investigate immunopathological mechanisms in cats with FEPSO and compare them with human VGKC encephalitis. We found that the cat brain shared many histopathological features with human VGKC-complex-Ab encephalitis including selective complement deposition in neurons and neuronal loss.

**MATERIALS AND METHODS**

**Animals**

Fifteen cats with clinical FEPSO and HN were included in this study. Except for 2 cats that died spontaneously, all animals were killed between 2 days and 34 months of onset of neurological signs because of resistance to therapy or severe clinical course. Therapy of cats consisted of standard antiepileptic treatment with phenobarbital, gabapentin, levetiracetam, or potassium bromide or combination. In addition, 4 cats were treated with prednisolone 1 to 2 mg/kg twice daily (Table). For controls, we used 3 cats with epileptic seizures that did not fit the classification of FEPSO (21) and hippocampal sclerosis. We also selected a group of cats with temporal lobe epilepsy displayed feline complex partial seizures with orofacial involvement (FEPSO) (21, 22). Our recent investigations revealed that many of these animals, in addition to facial involvement, have VGKC-complex/LGI1 Abs (23). Therefore, FEPSO in cats has much in common with human VGKC-complex-Ab encephalitis including facial-brochial dystonic seizures (14).

**Human Anti-VGKC Encephalitis**

For comparison with the human disease, we used brain tissue from a patient with LE (VGKC/2) with a high titer (958 pmol/L) of VGKC-complex-Ab described in our previous study (3). This case was chosen for 3 reasons. First, this case had the shortest disease duration (5 months). Second, whereas this case is autopsy material containing a complete cross section of the hippocampus, the other 3 cases were biopsies...
consisting of small pieces from the amygdala and uncus. Third (as seen in terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL] staining of all our cases), this case showed the most active neuronal degeneration associated with IgG and complement deposition.

**Magnetic Resonance Imaging**

Magnetic resonance imaging (MRI) was performed on 3 cats (Table) with FEPSO using a 1.5-T unit (high-field MRI; Magnetom Espree, Siemens Healthcare, Erlangen, Germany) in sternal recumbency under general anesthesia, with the head in a 15-channel transmit extremity coil. Imaging included transverse T2-weighted 3-dimensional spin echo images (remission time [TR] = 3000–5370 ms/ echo time [TE] = 111–388 ms) and T1-weighted 3-dimensional spin echo images (TR = 450–501 ms/TE = 12–17 ms), with multiplanar reformating features, transverse T2*-weighted gradient echo images (TR = 500 ms/TE = 39 ms; flip angle = 10 degrees), and transverse T2-weighted FLAIR (TR = 8500 ms/TE = 82 ms) images. T1-weighted images were re peated after intravenous administration of gadobenate dimeglumine (MultiHance; Bracco Österreich GmbH, Vienna, Austria) at a dosage of 0.15 mmol/kg body weight. Slice thickness was 0.8 to 3 mm, and slice separation was 0.1 to 0.8 mm.

**Serology**

Anti-VGKC Abs were assayed in 5 cats with FEPSO by routine immunoprecipitation of VGKC-complexes from rabbit brain extracts as described (5) and were applied for the diagnosis of LE in humans and previously implemented in cats (23). VGKC-complex-Ab positive sera were also tested for binding to LG1 and CASPR2 using cell-based assays (14). Abs to glutamic acid decarboxylase (GAD) were measured by radioimmunoprecipitation (RSR Ltd, Cardiff, UK). Feline-specific secondary Abs were used as appropriate.

**Neuropathology and Immunohistochemistry**

A general necropsy was performed in all animals. Brains were fixed in 4% neutral-buffered formalin, embedded in paraffin, transverse sectioned, and stained by hematoxylin and eosin and Nissl stains. Immunohistochemistry was performed as shown previously (3) at the level of the hippocampus using Abs against cat immunoglobulin (Jackson Immunoresearch, West Grove, PA), C9neo (rabbit-anti-rat; kind gift from S. Piddlesden, University of Cardiff, UK), T lymphocytes (anti-CD3; Labvision, Fremont, CA), a marker for microglia and macrophages (Iba-1; Wako, Osaka, Japan), and astrocytes (anti–glial fibrillary acidic protein; Dakopatts, Hamburg, Germany). Neurons were immunostained with an Ab to microtubule-associated protein 2 (MAP-2; Sigma, St. Louis, MO). To obtain information about cell death pathways in neurons, sections were stained for activated caspase 3 (detects caspase-dependent apoptosis in cells; CM1, Becton Dickinson, San Diego, CA).

**TUNEL**

Qualitative assessment of chronic cell loss was conducted in stained transversal sections at the level of the hippocampus. For the detection of cells with DNA fragmentation, TUNEL staining was performed with the In Situ Cell Death Detection Kit (Roche, Basel, Switzerland) according to Bien et al (3). This was followed by immunohistochemical staining for MAP-2 to identify dying neurons. TUNEL was developed with Fast Blue (Sigma); MAP-2 was developed with 3-amino-9-ethylcarbazole (Sigma) as a substrate.

**Quantitative and Semiquantitative Scoring**

Quantification of CD3-positive T cells, C9neo-positive neurons, and TUNEL-positive neurons was performed in hippocampal sections using an ocular morphometric grid covering an area of 1 mm² at 100× magnification. Numbers of counted cells were divided by the total volume of hippocampal area measured, leading to numbers of cells per squared millimeter (Table). For semiquantitative scoring of chronic neuronal loss in NeuN-stained hippocampal sections, the hippocampus was divided into 5 regions: CA1, CA2, CA3, CA4, and dentate gyrus. Neuronal loss was scored in each region, resulting in a

**FIGURE 1.** Magnetic resonance imaging (MRI), serology, and neuropathology of cats with feline complex partial cluster seizures with orofacial involvement (FEPSO). Numbers in parentheses indicate the animal number as designated in the Table. (A) T1 with contrast MRI of the brain showing an increase in T1 signal after contrast and an increase in volume of the hippocampi (arrowheads) and surrounding areas. (B–D) Enhanced green fluorescent protein expression of leucine-rich, glioma-inactivated 1 protein (LGI1)-transfected cells (B) and staining of antibodies from cat serum (C). Merged images (D) showing binding of cat immunoglobulins to LG1-expressing cells. (E–L) Double staining for deoxynucleotidyl transferase dUTP nick end labeling (TUNEL, blue) and microtubule-associated protein 2 (MAP-2)–positive neurons (red). (E) Hippocampus from a control cat showing dense MAP-2 immunoreactivity. (F) Higher magnification of (E) showing cells in the dentate gyrus (DG) and CA4 region of the hippocampus. (G) Hippocampus from a cat with FEPSO (Case 8) showing variable cell loss in various areas of the hippocampus. MAP-2–immunoreactive neurons are completely lost in the CA4 region. The rectangles indicated by 1 and 2 are shown at higher power in (H) and (I), respectively. (H) Higher magnification showing cell loss in the CA2 region of the hippocampus. Arrowheads point at TUNEL-positive (blue) nuclei of dying neurons. (I) Higher magnification of the CA3 region showing numerous TUNEL-positive neuronal nuclei and some remaining MAP-2–positive neurons and processes. (J–N) TUNEL and MAP-2 double staining of the hippocampus of another cat (Case 2) with FEPSO. (J) Distinct loss of neurons and fibers (arrowheads) in the dentate gyrus. (K) Enlargement of (J) showing numerous TUNEL-positive neuronal nuclei and loss of MAP-2–positive processes in the dentate gyrus and CA4 region. The rectangle indicates an area enlarged in (L) showing another higher magnification of numerous TUNEL-positive neurons in the dentate gyrus. (M) Hematoxylin and eosin staining showing neurons with eosinophilic cytoplasm and necrosis-like chromatin fragmentation into numerous speckles. (N) Caspase-3 staining in the degenerating hippocampal areas showing a small positive apoptotic cell (arrow) but absence of caspase-3 in neurons (arrowheads). The inset shows an enlargement of the apoptotic cell in which nuclear fragmentation (arrowhead) can be recognized. Scale bars = 1 cm (A); 100 μm (B–D, F, L); 1 mm (E, G, J); 50 μm (H, I); 250 μm (K); 100 μm (L); 10 μm (M); 20 μm (N).
cumulative score of 0 (no neuronal cell loss) to 5 (neuronal loss in all regions).

**Statistics**

Statistical evaluation was performed with Statgraph Prism 6. For the evaluation of numbers of CD3-positive T cells, C9neo-positive neurons, and TUNEL-positive neurons, a 2-sided Mann-Whitney U test was used. A p value of <0.05 was considered significant.

**RESULTS**

**Epileptic Seizures in Cats With FEPSO**

Seizures in cats were preceded by short episodes of unusual behavior with motionless staring (motor arrest), often in a sitting position. The seizures typically had an acute onset with confusion and characteristic orofacial signs such as facial twitching, excessive salivation, lip smacking, chewing, licking, and swallowing. Other signs observed included mydriasis, secondary generalized seizures, vocalization, and defecation. In addition, most cats showed behavioral changes such as fear or aggression. The 3 epileptic control animals (Table, Cases 16–18) demonstrated epileptic seizures not fitting FEPSO and other neurological signs such as circling and head tremor.

**MRI**

Only 3 animals had been screened by MRI. Cats with HN and clinical FEPSO-like epileptic seizures commonly show intense enhancement of T2 or T1 with contrast in the hippocampus and surrounding structures, as shown for Case 15 (Fig. 1A). Case 2 had a moderate bilateral increase of volume and T2 signal of the hippocampal region. The T1 signal was slightly decreased but increased after intravenous injection with gadobenate dimeglumine. Case 3 showed no abnormalities and bilaterally symmetric hippocampal volumes and signal.

**Serology**

Assays for Abs to VGKC-complex, the VGKC-complex proteins LGI1 and CASPR2, and GAD were performed in the 5 FEPSO cats with sera available. In 4 of these cases, VGKC-complex Abs were detected above the control range for humans (>100 pmol/L). Three of the 4 VGKC-complex Ab-positive cats also were positive for Ab to VGKC-complex protein LGI1 (Fig. 1B–D; Table). None of the 5 cats had anti-GAD or -CASPR2 Abs. The human VGKC encephalitis case used for comparison was positive for the anti-VGKC-complex Ab at a titer of 958 pmol/L, and the presence of anti-LGI1 or -CASPR2 Abs was not determined in this case (Table).

**Brain Pathology**

**Acute Neuronal Cell Death and Loss**

The human VGKC-complex encephalitis case (previously described in detail [3]) showed clear loss of neurons in all hippocampal subfields (Table). No gross lesions were evident in the brains of the 15 cats with FEPSO at necropsy. Histopathological examination demonstrated that lesions were present and usually distributed bilaterally in the hippocampus. In most cases, neuronal loss was evident in 1 or more of the 4 hippocampal subfields (Fig. 1G–I). In addition, a few cases showed degeneration and cell loss in the dentate granule cell layer (Fig. 1J–L). In single cases, extrahippocampal pathological changes were found in adjacent areas such as the entorhinal cortex, subiculum, parahippocampal gyrus, temporal cortex, basal ganglia, and diencephalon. Hippocampal neuronal degeneration was also present in all epileptic controls (Table). In addition, Case 16 of these controls showed degeneration in the neocortex. Neuronal degeneration was not evident in encephalitic and normal control animals (Table).

Acute neuron death was demonstrated by double staining for TUNEL and MAP-2. No TUNEL reactivity in the hippocampus was found in normal or encephalitis controls (Fig. 1E, F); however, 2 of 3 epileptic control cases showed the presence of TUNEL-positive neurons (Table). In the hippocampi of the 15 investigated FEPSO cases, there were on average 10 TUNEL-positive neurons/mm² hippocampus detected. This number of degenerating cells in FEPSO animals was comparable to the numbers of TUNEL-positive cells in the human VGKC-complex encephalitis patient (Table). The number of

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**FIGURE 2.** Immunopathology of feline voltage-gated potassium channel complex (VGKC-complex) encephalitis in cats with feline complex partial cluster seizures with orofacial involvement (FEPSO) and comparison with human VGKC encephalitis. (A) Staining for CD3 showing perivascular cuffs and parenchymal infiltration of T cells (arrowheads) in the hippocampus of a cat with FEPSO. (B) Perivascular cuff of the brain of a cat with FEPSO immunostained for CD3. There are CD3-positive and CD3-negative lymphocytes. (C) The same cuff as shown in (B) immunostained for CD20 showing that some of the lymphocytes are B cells (arrowheads). (D) Hippocampus of a patient with VGKC encephalitis stained for CD3 showing parenchymal infiltration of T cells. (E) Staining for lba-1 showing resting microglial cells in control (CON) cat hippocampus. (F) In a cat with FEPSO with neuronal damage lba-1-positive microglia are activated. (G) In a human VGKC encephalitis brain, microglia show activation and up-regulation of lba-1. (H–J) Staining for glial fibrillary acidic protein (GFAP) showing normal astrocytes in a control cat hippocampus, (H) while in the hippocampus of a cat with FEPSO, there is astrocyte activation and gliosis (I). There is also marked astrogliosis seen in the human VGKC encephalitis brain (J). (K) Immunostaining for cat immunoglobulin revealing the presence of plasma cells (arrowheads) in the perivascular cuffs of the brain of a cat with FEPSO. (L) Immunoglobulin staining of the dentate gyrus of a cat with FEPSO showing leakage of immunoglobulin in the parenchyma and uptake by neurons (arrowheads). (M) In some areas, immunoglobulin deposition is on neuronal membranes (arrowheads). (N) Similar membrane staining for immunoglobulin (black arrowheads) on neurons can also be found in the hippocampus of human VGKC encephalitis brain. In addition, the blue arrowheads points at a parenchymal plasma cell. (O–Q) Staining for C9neo. The hippocampus of a cat with FEPSO showing C9neo staining of almost all neurons of the CA1/CA2 region (O). In a higher magnification of the CA1 region, there is C9neo deposition in a granular pattern in neuronal cell bodies and processes (P, arrowheads). Staining of C9neo in the hippocampal neurons of a human VGKC encephalitis patient (Q, arrowheads) looks identical to the C9neo deposition of neurons in cat FEPSO hippocampus. Scale bars = 100 μm (A, J); 50 μm (B–I, N); 25 μm (K–M, P, Q); 200 μm (O).
TUNEL-positive cells in FEPSO animals did not differ from that in epileptic controls but was significantly higher than TUNEL-positive cells in the encephalitis group. Among the FEPSO animals, the extents of acute neuronal cell death were highly variable; some animals showed single TUNEL-positive neurons spread over the hippocampus, whereas others showed large numbers of TUNEL-positive neurons in distinct parts of the hippocampus (Fig. 1G–L). In all cases, TUNEL reactivity in neurons was associated with loss of MAP-2 immunoreactive processes (Fig. 1H). Degenerating neurons showed eosinophilic cytoplasm and chromatin fragmentation into fine speckles (Fig. 1M), as shown in ischemic rat hippocampi (24). Staining for the activated form of caspase-3 revealed the presence of small apoptotic cells, often with apoptotic bodies (Fig. 1N). Caspase-3 was not detected in neurons (Fig. 1N). In summary, although we cannot exclude the possibility that single neurons undergo apoptosis, the findings suggest that the bulk of degenerating neurons follow necrosis-like cell death rather than caspase-mediated apoptosis (24).

Neuron alterations in the FEPSO group were accompanied by moderate to severe astrogliosis as demonstrated by glial fibrillary acidic protein immunostaining (Fig. 2I); in all but 1 case, this was comparable to that in human VGKC encephalitis (3) (Fig. 2J). Astrogliosis was absent in control animals (Fig. 2H), but moderate to severe astrogliosis was also seen in the epileptic and encephalitic control groups.

Inflammation

CD3 immunohistochemistry in cats with FEPSO demonstrated that T cells comprised the majority of inflammatory cells. T cells were either localized in meninges only, in

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**FIGURE 3.** Complement C9neo expression in hippocampus of controls (CON), feline complex partial cluster seizures with orofacial involvement (FEPSO), and epileptic controls. Numbers in the panel labels indicate the animal number as shown in the Table. **(A)** A control animal showing no C9neo expression. **(B)** C9neo expression in the hippocampus of FEPSO cat. There is strong deposition in hippocampal CA2 and CA3 neurons (inset shows higher magnification). **(C)** In an epileptic control animal, there is a different (fiber-like) expression pattern around a focal inflammatory lesion. **(D)** In an epileptic control animal, there is intense C9neo deposition in the walls of blood vessels (bar = 200 μm). Insets in **C** and **D** show higher magnifications. Scale bars = 200 μm (**A–D**).
perivascular cuffs, and/or diffusely infiltrating the parenchyma (Fig. 2A, B). Parenchymal T-cell infiltration in most cases was moderate and on average (2.9 cells/mm² hippocampus) somewhat lower than the T-cell infiltration in the case of human VGKC encephalitis (13.2 T cells/mm² hippocampus) (3) (Table and Fig. 2D). Unexpectedly, T-cell numbers in the FEPSO cats were significantly lower than those in epileptic controls (p = 0.027) and did not differ from the encephalitic control cats (Table). No T cells in FEPSO animals were found in apposition to neurons.

Except for 1 cat with prominent perivascular infiltrates in the brain (and which displayed papillary adenomas in the lung (Case 1), tumors were not found in any cases. In addition to T cells, perivascular cuffs also contained CD20-positive B cells (Fig. 2C); immunoglobulin staining also showed moderate numbers of plasma cells (Fig. 2K). In all cases, most B cells and plasma cells were confined to the perivascular space; this was also the case in the epileptic and encephalitic controls. FEPSO animals (Fig. 2F), encephalitic and epileptic controls, and the human VGKC encephalitis case (Fig. 2G) all showed strongly activated microglia (i.e. with rounded morphology and shorter and thicker ramifications), as compared to normal feline controls (Fig. 2E).

**Immunoglobulin and Complement Deposition**

In normal animals, immunoglobulin staining was confined to the lumen of blood vessels (not shown). In FEPSO, epileptic, and encephalitic control animals, staining for immunoglobulin revealed substantial leakage through the blood-brain barrier (BBB). Diffuse immunoglobulin immunoreactivity in the parenchyma was seen on various cell types including neurons (Fig. 2L), astrocytes, and, in some cases, cells with oligodendrocyte or microglial cell morphology. Because of diffuse leakage of immunoglobulins, it was difficult to detect specific staining on the surface of neurons in cats with FEPSO. In some places, however, membranous deposition of immunoglobulins was present (Fig. 2M), which was also seen in the human VGKC-complex-Ab patient (Fig. 2N).

We have previously shown that human VGKC encephalitis cases show C9neo deposition in and on the surface of neurons (3). Here, in addition, we counted C9neo-positive cells in the hippocampus of selected VGKC encephalitis case; 4.4 cells/mm² are positive for C9neo (Table). As in human VGKC encephalitis, C9neo immunostaining revealed a punctate staining both inside and on the surface of neuronal cell bodies and their processes in FEPSO animals (Figs. 2O, P and 3B). This complement deposition was seen in 13 of the 15 FEPSO cases (on average, 8.6 positive neurons/mm²), including the 3 cats with Abs to LGI1 (Table and Fig. 3B). No deposition of C9neo on neurons was found in 2 remaining cases or in the encephalitic and normal controls (Fig. 3A and Table). In 2 of the 3 epileptic control cases (Cases 16 and 17), the C9neo staining pattern was also positive but located in different compartments and cells: Case 16 displayed strong C9neo immunostaining of the hippocampal parenchyma around a large inflammatory confluent lesion that mainly consisted of lymphocytes and large amounts of activated microglial cells/macrophages (Fig. 3C). Case 17 also showed marked inflammation, but C9neo was only present in the walls of blood vessels (Fig. 3D).

**DISCUSSION**

It has been recognized only recently that feline FEPSO, which often requires euthanasia, is likely to be pathogenically related to the form of human LE that occurs with VGKC-complex/LGI1 Abs. The presence of these Abs in a proportion of the cats (23) suggests that (like human patients) they may respond clinically to immunotherapies. In the meantime, the existence of postmortem tissue from these animals provides an opportunity to examine the pathology and to compare with the limited material available on the human disease. The results here demonstrate that, in most cases, the immunopathology is dominated by hippocampal neuron loss with relatively mild T cell infiltrates but with substantial IgG and complement deposition. These data suggest that FEPSO is an Ab-mediated disease and add support to the similar findings in the human disease.

HN in cats has been described in a number of studies. Supposed etiologies of this type of pathology include secondary epilepsy due to pyriform lobe oligodendroglioma (25), ischemia (26), anesthetic procedures (27), stroke (28), and toxins such as kainic acid (29), whereas causative agents such as feline leukemia virus and feline immunodeficiency virus infections were ruled out (16–18, 20). In many studies, the etiology of feline HN was not defined (16–18). Recently, we described a specific type of temporal lobe epilepsy with complex partial cluster seizures with orofacial involvement in cats (FEPSO) (21). The neuron loss and astrogliosis shown here resemble the pathology of feline HN described in the literature to date (16, 17, 20). In addition, we also demonstrate lymphocytic infiltrates in the hippocampus and other regions. These infiltrates contain not only T cells, but also numerous B cells and plasma cells, particularly in the perivascular cuffs. Thus, FEPSO animals have inflammation that is similar to the inflammation in human VGKC encephalitis (3, 30–32). It can be presumed that some of the idiopathic feline cases of HN reported previously might be associated with VGKC Abs.

In humans, LE has traditionally been considered to have a paraneoplastic etiology, but in the last 10 years, it has become clear that patients can have this condition in the absence of tumors, particularly when the LE is accompanied by Abs to the VGKC-complex proteins (5, 13). The most common tumors associated with LE in humans are small cell lung cancer and thymomas (5). Except for Case 1 with pulmonary adenomas, tumors have not been associated with feline HN. Therefore, it is unlikely that paraneoplastic processes are a major cause of the syndrome in cats. The fact that we could study a relatively large number of cases of FEPSO with HN, however, shows that this type of LE may (as in humans [5]) be quite frequent (5); thus, further testing of cats with these seizures could be informative.

Unfortunately, in our study, MRI was available from only 3 cats. At the time of MRI, the brain of Case 3 with FEPSO appeared normal, whereas the images of the other cats showed bilateral symmetric hippocampal T2 hyperintensity with contrast intake, indicative of inflammation. In humans,
MRI findings in VGKC-complex Abs and encephalitis typically demonstrate signal changes in the medial temporal lobes ranging from hippocampal swelling with T2/FLAIR signal increase to hippocampal atrophy. Usually later in the disease, with wider Ab testing, it has become clear that many patients do not show MRI changes at presentation (3, 5). In cats with FEPSO, hippocampal atrophy has not been demonstrated by MRI so far. Probably, this is because the limited anatomic resolution of hippocampal structures in cats makes changes of hippocampal size less evident.

Previous testing of sera from animals with FEPSO showed that, in the acute stage of their disease, 36% of these animals had IgG Abs to the VGKC-complex (23). Here, 5 FEPSO animals were available for serological testing, 4 of these were positive for VGKC-complex-Abs and 3 had LGI1 antibodies. The titers of anti-VGKC-complex Abs of these FEPSO cases ranged from 123 to 791 pmol/L, which is only slightly less than the titer in the human case. Therefore, findings in cats are comparable to human patients in whom titers of VGKC-complex Abs may fluctuate. Furthermore, not all Abs against the VGKC-complex also bind to the presently known specific antigens (i.e. LGI1, CASPR2, or Contactin-2) (23). This is also similar to human VGKC encephalitis, where in approximately 80% of the cases the Abs to the VGKC-complex do not bind to these antigens (13). Thus, it is likely that other antigens are part of the VGKC-complex and need to be identified in both cats and humans.

It has been shown that human VGKC encephalitis responds relatively well to immunotherapy such as hemapheresis or corticosteroid treatment (14, 15, 33–35). Moreover, preliminary evidence suggests that earlier therapy is related to better outcome (14, 34), and that more intense therapy combining immunosuppression with apheresis may be more effective than only monthly pulse methylprednisolone therapies (6, 15). In the present study, some cats were treated with corticosteroids but did not go into remission. Such treatments can, however, reduce VGKC-complex Ab titers (23); moreover, the treated cats in which Abs disappeared went into remission. The reason why some patients respond poorly or are left with clinical deficits (10) might be explained by the massive degeneration found in some brains. Both in human and cat brains, complement deposition on neurons was associated with massive neurodegeneration (3). Our results in cats show that loss of large numbers of degenerated hippocampal neurons can be detected within the first week of disease duration. This degeneration probably leads to functional deficits that cannot be reversed by lowering the Ab titers by immunotherapy. This is clearly different from NMDAR encephalitis in which no clear degeneration is found in most cases (3, 36); the Abs bind to NMDARs but do not seem to engage in complement-mediated destruction of neurons (3, 7, 37–39).

Neuronal loss and acute cell death in the FEPSO cats was accompanied by mild to moderate astrogliosis and activation of microglial cells. Both the chronic loss and the acute cell death detected by TUNEL were highly variable in the hippocampus but similar to that in humans (3). As in human VGKC encephalitis, FEPSO brains showed diffuse staining of immunoglobulin within neural cells, perhaps due to BBB leakage and uptake into neurons. However, in some areas, the immunoglobulin was detected on the surfaces of neurons, from which the IgG may be internalized as a result of cross-linking of the antigen, as is seen in other diseases such as myasthenia gravis (40) and NMDAR-Ab encephalitis (41). Most importantly, however, we detected C9neo staining, which indicates functional activation of the complement cascade, inside and on the hippocampal neurons of 13 of the 15 FEPSO cases. In particular, Case 1 (which was only of 5 days in duration), had strong C9neo deposition. On the other hand, for unknown reasons, Case 3 with a duration of 89 days and the highest VGKC-complex-Abs (including LGI1) did not show any hippocampal pathology. We hypothesize that Case 1 with low VGKC-complex-Abs and no identified serum Abs to LGI1 or CASPR2 provides a picture of the earliest stages of the acute disease as it develops and that further changes seen later in the other cases differ depending on factors that modify the natural history of the disease. Another possible explanation for the lack of correlation between serology and histopathology may be the different time of examination. Serology was usually carried out at the beginning of the disease and not just before the autopsy. In Case 3, more than 2 months passed between the start of acute LE and accompanying harvesting of serum, and despite corticosteroid treatment, there was development of hippocampal sclerosis and therapy-resistant epilepsy.

Overall, the presence of C9neo-positive neurons in the hippocampus of FEPSO animals is a specific feature that is shared with human VGKC-complex encephalitis (3) and clearly separates the FEPSO cases from the epileptic and encephalitic controls animals. The presence of C9neo-positive neurons also separates the VGKC encephalitis cases from other Ab-associated encephalitides such as NMDAR encephalitis (3).

An important question in Ab-associated encephalitides that is still unanswered is the question about the initiating event. Both in humans and in cats, VGKC-complex Ab encephalitis can present with fever (21), suggesting a prodromal infection (5, 13). Such a prodromal infection may, via the mechanism of molecular mimicry, generate a pathogenic Ab. However, so far, a specific infectious organism has not been identified in cats or humans, thus arguing against a direct role for molecular mimicry (1). Alternatively, infection with a viral or microbial agent may induce encephalitogenic T cells and trigger a systemic innate immune response that may be sufficient to disrupt BBB integrity (42, 43) and allow the efflux of the serum-predominant Ab into the CSF and brain parenchyma (44).

In conclusion, our findings may be helpful for improving diagnostic procedures such as serological testing and optimizing therapy in cats with FEPSO. In particular, immunotherapies may prove to be worthwhile and more effective than standard antiepileptic treatments. The similarities of FEPSO with the human disease extend to the immunopathology, in particular, to the specific presence of complement C9neo deposition on hippocampal neurons. As judged from an epilepsy surgery-based tissue bank (45), encephalitic brain lesions are a rare, specific cause of human epilepsies; similarly, in cats, the lesions described here are not a common finding in feline epilepsy. Further investigations in the nature of these specific well-defined conditions should therefore prove of benefit to the study of intractable seizures and LE in both feline and human patients.
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