Cell Proliferation and Granule Cell Dispersion in Human Hippocampal Sclerosis

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INTRODUCTION

Granule cell dispersion (GCD) of the hippocampus is a phenomenon uniquely observed in patients with epilepsy, typically in the context of hippocampal sclerosis (HS) (1–3). It appears to be related to severity of neuronal loss and is observed in 40% of surgical temporal lobe specimens (3). It has been suggested that GCD is due to excessive neurogenesis occurring as a result of seizures, as has been shown in animal models (4, 5), neuronal progenitor cell proliferation in patients with hippocampal damage and chronic epilepsy.

MATERIALS AND METHODS

Cases were selected from the pathology archives at the Division of Neuropathology, at the National Hospital for Neurology and Neurosurgery in London, England. The research has been approved by the Joint Research Ethics Committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery. All patients in the study had undergone temporal lobectomy, including hippocampal resection, for the treatment of intractable epilepsy between years 1994 and 1997. On routine neuropathologic examination, the severity of hippocampal damage was graded qualitatively, based on the severity of neuronal loss and gliosis as a modification of the Wyler score (9) as follows: Grade 1 = normal; Grade 2 = gliosis in end folium; Grade 3 = neuronal loss and gliosis in end folium; Grade 4 = neuronal loss and gliosis in both CA1 and CA4 with sparing of CA2 (classical HS); Grade 4a = neuronal loss and gliosis in CA1 only; Grade 5 = severe neuronal loss and gliosis in all subfields.

Fourteen patients were selected with a diagnosis of classical HS (Grade 4) and varying degrees of GCD as previously described (3). In 8 cases, there was very mild or insignificant dispersion and broadening of the granule cell layer (GCL). In 6 cases, severe dispersion of cells into the molecular layer (ML) with marked widening of the GCL was present, and in some of these cases, distinct clusters of granule cells were present in the ML. Six additional patients were included as a surgical control group in which an extra-hippocampal epileptogenic cortical lesion was present (LTLE). In these cases, the hippocampi showed overall milder degrees of neuronal loss (Grades 1–4a) compared with study cases. In addition, 5 postmortem hippocampi from patients with no history of epilepsy or other neurologic disease were selected as a further control group. The clinical and pathologic details of the epilepsy cases and controls are shown in Tables 1 and 2, respectively.

In all cases, a single representative paraffin block from each case was selected and further sections cut at 5 μm, dewaxed, and rehydrated in graded alcohol then washed in water followed by microwave in 0.05 mol/L EDTA, pH 7.5, for 15 minutes. Using DakoCytomation ChemMate diluent,
sections were incubated in primary antibodies; Mcm2 (BM 28 1:900, BD Biosciences, San Diego, CA) and Ki-67 (1:180, Dako, Glostrup, Denmark) for 2 hours at room temperature, followed by blocking of endogenous peroxidase for 10 minutes with Dako ChemMate hydrogen peroxide. Labeling was detected by using DaKo ChemMate Envision for 35 minutes and staining was visualized with NovaRed kit (Vector, Burlington, CA). Nuclei were counterstained with hematoxylin. The sections were dehydrated in alcohol, cleared in xylen, and coverslipped. Between each step, sections were washed with PBS containing 0.05% Tween 20. Anaplastic astrocytoma Grade III and normal human tonsil was used as positive control for Mcm2 and Ki-67, respectively. Negative controls were treated identically except that the primary antibody was replaced with normal mouse immunoglobulin IgG. In selected cases (Cases 2, 4, 10, and 11), immunohistochemistry for Geminin (using previously characterized polyclonal antibody G95 [10]) was carried out using a similar method. Semi-automated quantitative analysis of Mcm2-labeled sections was carried out using an image analysis system (Histometrix, Kinetic Imaging, Medical Solutions, Nottingham, UK). In each section, regions of the pyramidal cell layer of CA1, the GCL, ML of the dentate gyrus, and the hilar region (region between blades of GCL and including the subgranular cell layer [SGL]) were separately outlined at 2.5× magnification. Immunopositive cells were counted at a magnification of 40× (objective) within these regions in sequential nonoverlapping fields. The number of Mcm2-positive cells per mm² was then calculated for three regions in each case: region 1 (CA1); region 2 (GCL+ML); region 3 (hilus). Mean values between groups of cases were compared using ANOVA with SPSS software for Windows, version 10.

### TABLE 1. Clinical Information of Patients With Hippocampal Sclerosis With and Without Severe Granule Cell Dispersion

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Duration of Habitual Seizures (years)</th>
<th>Age at Surgery (years)</th>
<th>Sex</th>
<th>Initial Precipitating Injury</th>
<th>Side of Resection</th>
<th>Grade HS*</th>
<th>GCD, Mean Width (mm), Patterns of Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>39</td>
<td>M</td>
<td>Meningitis</td>
<td>Right</td>
<td>4</td>
<td>Mild 1.81</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>40</td>
<td>F</td>
<td>Unknown</td>
<td>Right</td>
<td>4</td>
<td>Mild 1.65</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>24</td>
<td>M</td>
<td>Prolonged FS</td>
<td>Right</td>
<td>4</td>
<td>Mild 1.94 Focal clusters</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>35</td>
<td>F</td>
<td>None</td>
<td>Left</td>
<td>4</td>
<td>Mild 1.16 Focal depletion</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>28</td>
<td>M</td>
<td>None</td>
<td>Left</td>
<td>4</td>
<td>Mild 1.33</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>25</td>
<td>F</td>
<td>None</td>
<td>Right</td>
<td>4</td>
<td>Mild 1.47</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>32</td>
<td>F</td>
<td>None</td>
<td>Left</td>
<td>4</td>
<td>Mild 1.28</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>28</td>
<td>F</td>
<td>Prolonged FS</td>
<td>Left</td>
<td>4</td>
<td>Mild 1.64</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>21</td>
<td>F</td>
<td>Unknown</td>
<td>Left</td>
<td>4</td>
<td>Severe 3.33 Diffuse</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>24</td>
<td>M</td>
<td>None</td>
<td>Right</td>
<td>4</td>
<td>Severe 3.21 Diffuse</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>34</td>
<td>F</td>
<td>None</td>
<td>Right</td>
<td>4</td>
<td>Severe 3.62 Diffuse</td>
</tr>
<tr>
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<td>NA</td>
<td>19</td>
<td>M</td>
<td>Unknown</td>
<td>Right</td>
<td>4</td>
<td>Severe 3.41 Clusters of GC</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>30</td>
<td>F</td>
<td>Prolonged FS</td>
<td>Left</td>
<td>4</td>
<td>Severe 2.9 Clusters of GC</td>
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<tr>
<td>14</td>
<td>47</td>
<td>48</td>
<td>F</td>
<td>Prolonged FS</td>
<td>Left</td>
<td>4</td>
<td>Severe 2.82 Focal bilayer</td>
</tr>
</tbody>
</table>

FS, febrile seizure; GCD, granule cell dispersion; NA, not available; HS, hippocampal sclerosis; GC, granule cells.

*Graded according to modified Wyler score. The mean width of the granule cell layer was calculated from the thickness in regions of maximal cell dispersion from three different measurements. Patterns of granule cell dispersion are recorded as diffuse, bilayer pattern or if there were distinct clusters of granule cells in the molecular layer.

### TABLE 2. Control Groups: Patients with Temporal Lobe Epilepsy and Extrahippocampal Lesional Pathology (LTLE) (Cases 1–6) and Postmortem Controls (Cases 7–11)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at Surgery or Death (years)</th>
<th>Sex</th>
<th>Side of Pathology</th>
<th>Pathology or Cause of Death</th>
<th>Grade HS*</th>
<th>Postmortem Interval and Fixation Time</th>
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<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>F</td>
<td>Right</td>
<td>Old infarct</td>
<td>4a</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>F</td>
<td>Left</td>
<td>Old scar</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>M</td>
<td>Right</td>
<td>Old infarct</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>F</td>
<td>Left</td>
<td>Old infarct</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>F</td>
<td>Left</td>
<td>Old infarct</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>F</td>
<td>Left</td>
<td>Ganglioglioma</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>M</td>
<td>Right</td>
<td>IHD</td>
<td>1</td>
<td>1 day; 1 week</td>
</tr>
<tr>
<td>8</td>
<td>69</td>
<td>F</td>
<td>Right</td>
<td>IHD</td>
<td>1</td>
<td>3 days; 1 week</td>
</tr>
<tr>
<td>9</td>
<td>77</td>
<td>M</td>
<td>Left</td>
<td>IHD</td>
<td>1</td>
<td>4 days; 1 week</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>M</td>
<td>Left</td>
<td>Pulmonary embolus</td>
<td>1</td>
<td>3 days; 1 week</td>
</tr>
<tr>
<td>11</td>
<td>85</td>
<td>F</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
<td>2 days; 5 weeks</td>
</tr>
</tbody>
</table>

HS, hippocampal sclerosis; NA, not applicable or available; IHD, ischemic heart disease.

*Graded according to modified Wyler score.
In sections from Cases 2, 7, and 13, double labeling immunohistochemistry for Mcm2/GFAP or Geminin/GFAP was carried out. Five-micron paraffin embedded sections were dewaxed, rehydrated through graded alcohols, and taken to water. Endogenous peroxidase was blocked with 1% hydrogen peroxide in methanol for 15 minutes. Sections were washed and microwaved for 15 minutes in 0.05 mol/L EDTA, pH 7.5, and cooled for 20 minutes. Protein blocking was done by incubating sections in 10% normal swine serum for 20 minutes. Antibodies to Mcm2 (1:1,000, BD Biosciences), or Geminin G95 (1:1,000) were applied overnight at 4°C. Labeling was detected using Dako LSAB Plus kit, staining was visualized by DAB + Nickel (Vector). After washing, sections were soaked in 1% hydrogen peroxide for 10 minutes. Sections were incubated with GFAP (1:3000 Dako) for 1 hour at room temperature. Labeling was detected with DAKO LSAB Plus kit, and staining was visualized by NovaRed (Vector). Sections were dehydrated, cleared, and mounted. Antibodies were diluted in Dako ChemMate diluent and all washes were done with PBS containing 0.05% Tween 20.

Similarly, for immunofluorescence labeling, 5-µm-thick, paraffin-embedded sections from selected HS cases (2, 7, 11–13) were dewaxed, rehydrated through graded alcohols, and taken to water. Sections were microwaved for 15 minutes in 0.05 mol/L EDTA, pH 7.5, and cooled for 20 minutes. Sections were incubated with either polyclonal antibodies to GFAP (1:300), S-100 (1:1000 dilution, Dako), nestin (1:600 Chemicon, Temecula, CA), calretinin (1:1000 Swant, Bellinzona, Switzerland), dynorphin (1:20 Serotec, Oxford, UK), and monoclonal Mcm2 (1:300, Isotype IgG1, BD Biosciences) or to Mcm2 as above with monoclonal CD68 antibody (1:100, Isotype IgG3, Dako). All antibodies were diluted in Dako ChemMate antibody diluent and sections were incubated at 4°C overnight. Sections were washed with PBS containing 0.05% Tween 20. Sections were incubated with the mixture of Alexa Fluor 594 Chicken anti-rabbit (1:100) and Alexa Fluor 488 Goat anti mouse (1:100 Molecular Probe, Leiden, Netherlands) diluted in PBS containing 1% BSA and 0.05% Tween 20 overnight at 4°C. For the two monoclonal antibodies, Alexa Fluor isotype specific secondaries were used. Sections were washed and mounted on Vectashield with DAPI and visualized with a Zeiss fluorescence microscope and a Leica DMRE-SP2 (MP-UV) confocal microscope.

**RESULTS**

In all HS and LTL cases, Mcm2-positive cells were present in the hippocampus, particularly in the region of the dentate gyrus, including the GCL and ML (Fig. 1a–c). The morphology of the labeled cells was mainly that of small cells with densely stained round-ovoid nuclei with little discernible cytoplasm. Occasional cells with the appearance of a granule cell were labeled in the ML and where clusters of dispersed granule cells were present in the ML, positive cells among these groups were seen (Fig. 1b). Both the supra- and infrapyramidal blades of the GCL contained immunopositive cells without an apparent difference, although this was not quantitatively assessed. No mature pyramidal neurons were labeled in any region. There were also Mcm2-positive cells in the hilus, CA1 sector, subiculum, and entorhinal cortex in epilepsy cases. In postmortem controls, Mcm2-positive cells were also seen in these subregions, albeit fewer in number. In epilepsy cases using Ki-67 immunohistochemistry, only occasional positive cells of similar morphology to Mcm2-positive cells were seen in the region of the GCL (Fig. 1d).

Quantitative analysis showed similar densities of Mcm2-positive cells in the CA1 region in control groups and HS cases with mild or severe GCD (Table 3; Fig. 2). There were significantly higher numbers of Mcm2-positive cells in the GCL and hilus in HS cases with severe dispersion compared with other groups (p < 0.05). In patients where clinical data were available (Tables 1, 2), there was no correlation between Mcm2-positive cell densities and the age of patient at surgery, duration of seizures, or the presence of an initial precipitating injury, such as a febrile seizure. There was a correlation between the grade of HS and the Mcm2-positive cell density in region 2 (GCL and ML; Spearman correlation, p = 0.039). The distribution and morphology of cells labeling with anti-Geminin antibody were similar to Mcm2 immunohistochemistry (Fig. 1g). Although these sections were not quantitatively assessed, the impression was that fewer positive cells were seen labeled for Geminin.

In cases studied with double labeling for Mcm2/GFAP and Geminin/GFAP, the majority of Mcm2- or Geminin-positive cells in the dentate gyrus were GFAP-negative (Figs. 1f, g). In most HS cases, there were dense meshworks of glial fibers with a predominantly radial arrangement in the GCL and ML with GFAP immunohistochemistry. Interspersed astrocytic cell bodies were seen, some with a more stellate morphology (Fig. 3a, b). In many cases, Mcm2-positive nuclei were in close proximity to such radial glial fibers, but an origin of fibers from these cells in most cases could not be confirmed. With anti-CD68 double labeling, occasional colocalization with Mcm2 was observed (Fig. 4c, d), but the majority of Mcm2-positive cells in the GCL were CD68-negative (Fig. 4a, b). In one autopsy control (Case 7), several Mcm2-positive cells in the hippocampal subregions showed morphology compatible with microglia. Dynorphin labeled the cytoplasm of GC intensely in 2 of the 3 cases (11 and 13), but colocalization with Mcm2 was not seen (Fig. 4e). Similarly, there was no colocalization of calretinin with Mcm2 (Fig. 4f) and S-100 labeled very occasional Mcm2-positive cells in the GCL (Fig. 4g). Colocalization of nestin and Mcm2 was demonstrated in 2 cases (2 and 12) both without and with GCD (Fig. 3c–f). In these cases, the double-labeled cells were located in the infranuclear and supra-granular cell layers, were infrequent in number, and were morphologically similar to cells observed using conventional nestin immunohistochemistry (Fig. 1).

**DISCUSSION**

GCD is observed in approximately 40% of surgical specimens of hippocampal sclerosis in patients with epilepsy and appears unique to this condition (1–3). It has been considered to be a developmental abnormality (1, 11), or the result of the effect of early seizures on a maturing hippocampus. We have previously shown that the severity of GCD may vary both within and between HS cases, but there appears to be some
correlation with the degree of loss of principal neurons in the hippocampus (3).

In the mature hippocampus, it is well recognized that there is continued renewal of GC from progenitor cells located in the subgranular cell zone or layer (SGL) (12, 13). This process is likely to have important physiological implications for the plasticity of this region, for example, in relation to memory and mood (14). In experimental conditions, brain insults, including seizures, have been shown to alter the rates of GC renewal. For example, in adult rats, increased neurogenesis and migration are seen following pilocarpine-induced status epilepticus (4) and kainic acid-induced seizures (15). It is highly plausible, therefore, that GCD in HS is a manifestation of increased neurogenesis as a result of seizures. This was supported by our previous quantitative studies showing an overall loss of GC in HS compared with controls, but increased

FIGURE 1. Hippocampal sclerosis with severe granule cell dispersion (GCD) with Mcm2 immunohistochemistry. In Case 12 (a, b), nests of displaced granule cells are seen in the molecular layer (arrow indicating cluster shown in b) and frequent positive cells in the granule cell layer, molecular layer and hilus are seen. In Case 10 (c, d), numerous cells in the region of the dispersed granule cell layer are positive with Mcm2 (c) whereas with Ki-67 in the corresponding region (d), only occasional positive nuclei are seen. (e) Nestin-positive cell in Case 12 in the subgranular cell layer in HS (arrow). (f, g) Hippocampal sclerosis with marked gliosis in the granule cell layer (Case 2). Double labeled immunohistochemistry with (f) GFAP and Mcm2 and (g) GFAP and Geminin (G95) antibody, both show positive labeled nuclei which are not GFAP-positive (dark arrow), compared to reactive astrocytes in the region (open arrow). Bars: (a) = 90 μm; (b–d) = 45 μm; (e, f) = 20 μm; (g) = 15 μm.
cell numbers in regions of GCD (3). Further in support of this theory, nestin-positive neuronal precursor cells have been demonstrated in the dentate gyrus of young patients with HS (16); and in the present study, we have demonstrated smaller numbers of similar nestin-positive cells in adults with HS.

In experimental systems, BrdU labeling has been used for the identification of newly born cells. This is rarely feasible in human tissues and conventional cell proliferation markers such as Ki-67 and cyclins have previously been used in the study of HS (16–18). Small numbers of immunopositive cells have been seen in the dentate gyrus, suggesting neurogenesis in the adult human hippocampus. In the present study, we used the cell cycle marker Mcm2, a DNA replication-licensing factor. Mcm2 expression is a novel marker of cell turnover that detects not only cycling cells but also cells with proliferative potential (6) and is expressed throughout all phases of the proliferative cell cycle (G1-S-G2-M) (7). Withdrawal of cells from the proliferative cell cycle into quiescent (G0), senescent, or terminally differentiated “out of cycle” states is coupled to downregulation of the Mcm replication licensing proteins (6, 7, 19). We have shown previously that loss of proliferative capacity in mature adult neurons is coupled to downregulation of the MCM proteins (6, 7). Mcm2 therefore detects a larger growth fraction than Ki-67 and has been used to identify slowly dividing neuronal stem cells as well as more rapidly dividing progenitor cell in rodent brain (8). In the present study, greater numbers of positively labeled cells in the granule cell layer were detected with Mcm2 than Ki-67 immunohistochemistry. Geminin, which acts as a repressor of origin licensing, detects a smaller growth fraction than Mcm2 due to its restricted expression to the S-G2-M phases of the cell cycle (7, 10). The confirmation of Geminin- and Ki-67-labeled cells in lower numbers than Mcm2-labeled cells is evidence that a subset is progressing through the proliferative cell cycle (10).

In HS cases with severe GCD, we observed higher numbers of Mcm2-positive cells in the dentate gyrus, the mean number being 16.4/mm². Previous quantitative studies of similar anatomic regions in HS patients with the Ki-67 marker showed densities of 1.0 to 1.5/mm² (16), these differences reflecting detection of more cells with proliferative potential by Mcm2. Examination of a postmortem control group, with an older age range than the epilepsy patients (Table 2), confirmed basal levels of expression of Mcm2 in the hippocampus. We noted, however, similar densities of Mcm2-positive cells in the CA1 region in HS patients, LTLE patients, and controls. This suggests the different densities noted in the GCL between groups are a genuine observation and related to the severity of the GCD.

The nature of the cycling cells was investigated using double labeling and confocal microscopy. The morphology of Mcm2-positive cells in the GCL region was predominantly that of small, immature-appearing cells with hyperchromatic nuclei and little discernible cytoplasm that were mostly immunonegative for GFAP, CD68, S-100, calretinin, and dynorphin. Dynorphin expression by mature GC is observed in the majority of adult HS specimens (20), whereas calretinin is transiently expressed by immature postmitotic granule cells (13). There is typically extensive reactive gliosis of this region in HS, with extension of radial processes into the molecular layer, and it has been noted in animals studies that it is often difficult to resolve with certainty whether the GFAP-positive processes in this region are arising from the proliferating cells or enveloping them. Interestingly, it has recently been demonstrated that astrocytic or radial glial cells are indeed the primary precursor cells for new neurons in the dentate gyrus (21). Furthermore, following seizures, preferential proliferation of radial glia in the dentate gyrus has been shown (22). In rodents, a further subtype of GFAP-negative cell, the “D” cell, with dense nuclei, has also been recognized, representing transient neuronal precursors (21, 22). Morphologically, the Mcm2-positive cells in our study are reminiscent of such D cells or transient neuronal precursor cells (13). The demonstration of the stem cell marker nestin in a small number of Mcm2-positive cells in the GC layer is evidence that some of these cells are immature or precursor cells of potential neuronal lineage. The mechanisms regulating neurogenesis may be influenced by both seizures and cell death occurring in the dentate gyrus regions but not CA1 than other groups.

**FIGURE 2.** A box plot illustrating the mean number of Mcm2-positive cells in three hippocampal subregions, CA1, hilus, and dentate gyrus (granule cell layer and molecular layer) in HS cases with and without severe granule cell dispersion (GCD), lesional temporal lobe epilepsy (LTLE), and postmortem (PM) controls. Significantly more immunopositive cells were seen in the cases with severe granule cell dispersion (in the CA4 and dentate gyrus regions but not CA1) than other groups.
FIGURE 3. Double-labeled immunofluorescence for GFAP (Alexa 594, red) and Mcm2 (Alexa 488, green) in hippocampal sclerosis (Case 13) with severe granule cell dispersion. Confocal imaging shows thick interlacing meshworks of radial glial fibers are seen but the majority of Mcm2-positive cells appeared not to be GFAP-positive compared with identified mature astrocyte with stellate morphology in this field (a, arrow). In Case 2, with mild dispersion and similarly labeled, Mcm2-positive nuclei are seen in the subgranular and granule cell layer (b, arrows), but in this field these cells are not GFAP-positive. (c–f) Nestin immunohistochemistry in Case 12 (c, e) and Case 2 (d, f) showing occasional Mcm2-positive cells in the subgranular cell layer, which also show cytoplasmic labeling with nestin antibodies ([c] and [d] shown with DAPI nuclear stain and [e] and [f] are corresponding images with Mcm2 nuclear stain alone) on confocal imaging. Bars: (a), (c–f) = 10 μm; (b) = 20 μm.
epilepsy patients. Studies in animal models have implicated neuropeptide Y in the regulation of neurogenesis (23). This may be relevant in HS as changes in NPY-expressing neuronal populations are known to occur (20).

Mcm2-labeled cells in HS cases, particularly those with GCD, were not restricted to the subgranular zone, compatible with the view that there is migration of dividing cells into the ML. Ectopic localization of nestin-positive precursor neurons in the ML has been previously shown in young children with TLE (16). In the pilocarpine model of status, newly born neurons were also shown to have widely migrated, with some present in the hilus, ML (4), and in CA3 region (5). Although in human tissues, GCD is a phenomenon that is peculiar to patients with seizures or malformations, in animal models it has been observed following other forms of brain injury (24). The molecular signals causing dispersion are unknown but may be related to those controlling dentate gliogenesis and neurogenesis. Indeed, immunophenotypic immaturity of astrocytes has been shown in HS cases with more severe GCD (25).

In some animal models of malformation, such as the p35 knock-out and reeler mutant, abnormal dispersion of GC is a striking feature (26, 27), which may be due to disruption of the radial glial scaffold (28). In human temporal lobectomies, an inverse correlation between the density of reelin-synthesizing cells and the severity of dispersion has been shown in some but not all studies (3, 28), implicating the reelin pathway in GCD. It seems likely from these studies that radial glia play a key role in both regulation of proliferation and migration of granule cells.

It has been shown experimentally that 50% of newly generated neurons in GCL will have prolonged survival and are likely to be functionally integrated into hippocampal networks (29). Cell proliferation in animal models has been shown to peak 3 days following a seizure (22) and then decline, but it is unknown how often precursor cells divide before giving rise to fully differentiated neurons (21). The integration of any new neurons locally is key to their potential contribution to epileptogenic circuits. Functional integration of embryonic stem cells introduced into hippocampal slices has been demonstrated experimentally, highlighting the capabilities of “new” neurons (30). In this study, we have shown potentially more proliferation in cases with severe GCD compared to those with less dispersion. As both these groups had chronic TLE due to HS, indistinguishable in clinical severity, it calls into question the relative contribution of “new” neurons to epileptogenesis. It is possible that the severity of GCD represents different stages in the evolution of HS and over time, the rate of any generation of new cells may be counterbalanced by neuronal loss and potential depletion of the precursor pool. In addition, there is a reduced capacity for neurogenesis with increasing age. In the present study,

**FIGURE 4.** Case 11 Mcm2 labeling (Alexa 594, red) combined with CD68 (Alexa 488, green) (a, c) and CD68 alone in identical corresponding regions (b, d) in the granule cell layer in HS. The majority of Mcm2-positive cells do not colocalize with CD68 (arrows) with only occasional cell showing positivity with both markers in the granule cell layer (c, d). Confocal images; Mcm2 immunofluorescence (Alexa 488, green) combined with (Alexa 594, red) (e) dynorphin immunohistochemistry (Case 13) (f) calretinin (Case 12), and (g) S-100 (Case 12) showing no colocalization of Mcm2 with dynorphin- or calretinin-positive cells and occasional cells colocalizing with S-100, which also demonstrated the intense gliosis in this region (f). Bars: (a–d), (f), and (g) = 25 μm; (e) = 30 μm.
however, we found no correlation between the age of the patient and controls and the numbers of Mcm2-positive cells in any hippocampal region. There is also speculation that neurogenesis may occur in other regions of the adult brain, including neocortex (31), possibly from “germinal astrocytes” or other progenitor cells (32). As the proliferation of such cells may also be influenced by seizures, this may have wider and important implications for our future understanding of the cellular mechanisms of focal epilepsies.

REFERENCES