Immunohistochemical Expression of Prion Protein (PrPC) in the Human Forebrain During Development

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Abstract

The cellular prion protein (PrPC) is a ubiquitous protein whose expression in the adult brain occurs mainly in synapses. We used monoclonal antibodies to study fetal and perinatal PrPC expression in the human forebrain. Double immunofluorescence and confocal microscopy with GFAP, Iba1, MAP2, doublecortin, synaptophysin, and GAP-43 were used to localize PrPC. PrPC immunoreactivity was observed in axonal tracts and fascicles from the 11th week to the end of gestation. Synapses expressed PrPC at increasing levels throughout synaptogenesis. At midgestation, a few PrPC-labeled neurons were detected in the cortical anlage and numerous ameboid and intermediate microglial cells were PrPC-positive. In contrast, at the end of gestation, microglial PrPC expression decreased to almost nothing, whereas neuronal PrPC expression increased, most notably in ischemic areas. In adults, PrPC immunoreactivity was restricted to the synaptic neuropil of the gray matter. At all ages, choroid plexus, ependymal, and endothelial cells were labeled, whereas astrocytes were only occasionally immunoreactive. In conclusion, the early expression of PrPC in the axonal field may suggest a specific role for this molecule in axonal growth during development. Moreover, PrPC may play a role in early microglial cell development.

Key Words: Axonal growth cones, Brain, Cellular prion protein, Fetus, Microglia, Neuron, Synapse.

INTRODUCTION

The cellular prion protein (PrPC) is a glycosylphosphatidylinositol (GPI)-anchored membrane sialoglycoprotein that is found in many tissues, including the brain. In prion disease, posttranslational events convert PrPC to the protease-resistant conformer PrPsc, which accumulates in the tissue. PrPC gene expression has been documented in neurons (1–4), and PrPC protein has been found chiefly in lipid rafts (5) and synapses (6, 7). In addition, PrPC labeling of the neuronal perikaryon has been reported (4, 8, 9).

Most studies of PrPC expression during development were conducted in rodents (10–15). Researchers detected PrPC mRNA by RT-PCR in mouse embryos at midgestation (15) and PrPC protein by Western blot in perinatal rodent brain (14, 15). Immunohistochemistry, which has been used only in postnatal animals (14), showed PrPC in the axonal field and synaptic compartments but not in the cell bodies. A single study investigated the perinatal expression of PrPC protein in humans (16). Information on prenatal PrPC expression in the human brain might provide new insight into the functions of this protein, which remain largely unknown. PrPC may be involved in cell signaling (17, 18), cell adhesion (19), neurite outgrowth, cell death or survival (20–22), regulation of oxidative stress (23), and synaptic function (24).

The objective of our study was to investigate prenatal PrPC expression in the human telencephalon. We investigated the distribution of PrPC in various cell compartments of the human forebrain during development. Prion protein labeling was combined with several cell markers to establish the cell types expressing PrPC in numerous fetal brains spanning a broad range of gestational ages.

MATERIAL AND METHODS

Brains

Postmortem brains were obtained from 25 fetuses aged 11 to 39 gestational weeks (gw), determined as weeks postovulation, and from one adult (Table 1). The fetal brains were from the products of spontaneous or therapeutic abortions and were free of detectable neuropathologic abnormalities, except for mild to moderate ischemia–anoxia in 2 of the perinatal brains. Tissue procurement procedures were consistent with the protocol approved by the French National Ethics Committee (CCNESVS). Tissue fixation was started within 24 hours after death. The brains were placed in formalin for 1 week, and dissected blocks were then embedded in paraffin. For some brains, one hemisphere was
Immunohistochemistry

Both cryostat and paraffin sections were used for this study. Paraffin-embedded blocks were cut into 5-μm sections and deparaffinized. Endogenous peroxidase was blocked using 3% H₂O₂. Antigen retrieval was performed using pH 6 target retrieval buffer (DakoCytomation) in a water bath for 40 minutes and in phosphate-buffered saline containing 0.5% Triton X and 0.2% gelatin. Sections were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer and processed for cryostat sectioning (25).

TABLE 1. Summary of Paraffin-Embedded and Frozen Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Specimen</th>
<th>Age (gestational weeks)</th>
<th>Cause of Death</th>
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<tbody>
<tr>
<td>1</td>
<td>Paraffin</td>
<td>11</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>2</td>
<td>Paraffin</td>
<td>12</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>3</td>
<td>Paraffin</td>
<td>12</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>4</td>
<td>Paraffin</td>
<td>12</td>
<td>Induced abortion</td>
</tr>
<tr>
<td>5</td>
<td>Frozen</td>
<td>13</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>6</td>
<td>Paraffin</td>
<td>15</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>7</td>
<td>Paraffin</td>
<td>16</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>8</td>
<td>Frozen</td>
<td>16</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>9</td>
<td>Paraffin</td>
<td>18</td>
<td>Induced abortion</td>
</tr>
<tr>
<td>10</td>
<td>Paraffin</td>
<td>18</td>
<td>Induced abortion</td>
</tr>
<tr>
<td>11</td>
<td>Paraffin</td>
<td>18</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
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<td>19</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>13</td>
<td>Frozen</td>
<td>19</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>14</td>
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<td>20</td>
<td>Spontaneous miscarriage</td>
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<td>Paraffin</td>
<td>20</td>
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</tr>
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<td>16</td>
<td>Paraffin</td>
<td>20</td>
<td>Induced abortion</td>
</tr>
<tr>
<td>17</td>
<td>Paraffin</td>
<td>20</td>
<td>Induced abortion</td>
</tr>
<tr>
<td>18</td>
<td>Paraffin</td>
<td>23</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>19</td>
<td>Paraffin</td>
<td>25</td>
<td>Induced abortion</td>
</tr>
<tr>
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<td>21</td>
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<tr>
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<td>37</td>
<td>Spontaneous miscarriage, ischemia</td>
</tr>
<tr>
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<td>Frozen</td>
<td>38</td>
<td>Spontaneous miscarriage</td>
</tr>
<tr>
<td>25</td>
<td>Paraffin</td>
<td>39</td>
<td>Spontaneous miscarriage, ischemia</td>
</tr>
<tr>
<td>26</td>
<td>Paraffin</td>
<td>30 years</td>
<td>Viral disease</td>
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TABLE 2. Antibodies Used in the Study

<table>
<thead>
<tr>
<th>Antibody (PrPC epitopes)</th>
<th>Isotype</th>
<th>Dilution (paraffin)</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>BG4 (23–85)</td>
<td>IgG2b</td>
<td>1/1000</td>
<td>TSE Resource Center (Compton, U.K.)</td>
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<tr>
<td>3F4 (109–112)</td>
<td>IgG2a</td>
<td>1/800</td>
<td>Senetek (Maryland Heights, MO)</td>
</tr>
<tr>
<td>6H4 (144–152)</td>
<td>IgG1</td>
<td>1/500</td>
<td>Prionics (Zürich, Switzerland)</td>
</tr>
<tr>
<td>12F10 (142–160)</td>
<td>IgG2a</td>
<td>1/1000</td>
<td>Cayman Chemicals (Ann Arbor, MI)</td>
</tr>
<tr>
<td>KG9 (140–180)</td>
<td>IgG1</td>
<td>1/1500</td>
<td>TSE Resource Center</td>
</tr>
<tr>
<td>MAP2</td>
<td>IgG1</td>
<td>1/1000</td>
<td>Sigma (St. Louis, MO)</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Rabbit</td>
<td>1/50</td>
<td>Dako (Glostrup, Denmark)</td>
</tr>
<tr>
<td>Doublecortin</td>
<td>Guinea pig</td>
<td>1/3000</td>
<td>Chemicon (Temecula, CA)</td>
</tr>
<tr>
<td>CaBP</td>
<td>rabbit</td>
<td>1/3000</td>
<td>Swant (Bellinzona, Switzerland)</td>
</tr>
<tr>
<td>Calretinin</td>
<td>rabbit</td>
<td>1/3000</td>
<td>Swant</td>
</tr>
<tr>
<td>GAP-43</td>
<td>Rabbit</td>
<td>1/200</td>
<td>Gift of Prof. F. Margolis, Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD</td>
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<tr>
<td>GAP-43</td>
<td>IgG2a</td>
<td>1/2000</td>
<td>Sigma</td>
</tr>
<tr>
<td>GFAP</td>
<td>Rabbit</td>
<td>1/500</td>
<td>DAKO</td>
</tr>
<tr>
<td>Iba1</td>
<td>IgG1</td>
<td>1/1500</td>
<td>Gift of Pr. Y. Imai, Department of Neurochemistry, National Institute of Neuroscience, Kodaira, Tokyo, Japan</td>
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</tbody>
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fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer and processed for cryostat sectioning (25).

another microtubule-associated protein, is expressed in migrating and differentiating neuronal cells until midgestation. The calmodulin-binding phosphoprotein GAP-43 (growth-associated protein-43) is abundant in elongating neurites and growth cones. In adults, GAP-43 is attached to the presynaptic membrane and may be involved in establishing and remodeling synaptic connections. Calretinin and Calbindin-D28k are calcium-binding proteins expressed in differentiating cell bodies and in adult interneurons. Labeling was visualized using the streptavidin–biotin–peroxidase method (Amersham, Arlington Heights, IL) with DAB-nickel as the chromogen (25). Synaptophysin immunostaining was performed using Immunostainer NexES (Ventana Medical Systems Inc., Illkirch, France). Double Immunofluorescence and Confocal Microscopy

For double labeling, higher concentrations of primary antibodies were used. The fluorescent-labeled secondary antibodies were Alexa Fluor 488 goat antiguinea pig IgG, Alexa Fluor 546 goat antiguinea pig IgG, Alexa Fluor 488 goat antirabbit IgG, Alexa Fluor 546 goat antirabbit IgG, and Alexa Fluor 546 goat antiguinea pig IgG (Fluoroprobes, Interchim Montlucon, France).
France). Double labeling with isotype-specific monoclonal antibodies was performed using the Zenon Alexa Fluor 488 mouse IgG2a labeling kit (12F10 labeling) and the Zenon Alexa Fluor 546 mouse IgG1 labeling kit (MAP2 labeling) (Molecular Probes, Eugene, OR). Slides were examined using confocal laser scanning microscopy (LSM-510-META microscope; Zeiss, Oberkochen, Germany) and LEICA DMIRB videomicroscopy (Leica, Rueil-Malmaison, France).

### TABLE 3. A Global Overview of PrPC Labeling in Different Areas and Cellular Compartments in Developing Telencephalon

<table>
<thead>
<tr>
<th>Stages gestational weeks</th>
<th>Specimens</th>
<th>‘Subplate’ Intermediate zone</th>
<th>White Matter</th>
<th>Cortical Plate</th>
<th>Cortex</th>
<th>Synapses</th>
<th>Neurons</th>
<th>Microglia</th>
<th>Vessels</th>
<th>Choroid Plexus</th>
<th>Ependyma</th>
<th>Astrocytes</th>
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<tr>
<td>11–23</td>
<td>P</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25–32</td>
<td>F</td>
<td>+++</td>
<td>++</td>
<td>+/-</td>
<td>+/-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>33–39</td>
<td>P</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>+/-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adult</td>
<td>F</td>
<td>+/−</td>
<td>+/-</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Growing axonal tracts develop and express PrPC earlier in the subplate and intermediate zone than in the cortical plate. PrPC-labeled neurons were inconsistently observed and often distributed in patches. Microglial cells strongly expressed PrPC at midgestation (they were inconsistently labeled in paraffin sections). Microglial PrPC expression decreased gradually from midgestation onward; however, it was present in some perivascular macrophages in the adult case.

P, paraffin-embedded specimens; F, frozen specimens; 0, no labeling; +, faint labeling of tracts and synapses or few cells; ++++, strong labeling of tracts and synapses or numerous cells.

**FIGURE 1.** PrPC immunoreactivity (PrPC-IR) until midgestation. (A) At 11 gestational weeks (gw), PrPC labels the fornix (F), internal capsule (IC), and fiber tracts running in the subplate under the cortical plate (CP). (B) PrPC labeling parallels GAP-43 immunoreactivity. (C–H) At 18 gw, PrPC-IR (C) is more intense, no labeling is seen in the ventricular zone, and dense fiber tracts are labeled within the pallidum (Pa) compared with the putamen (P). SP, subplate. (D–H) PrPC-IR (D) parallels GAP-43 labeling (E) and is observed (D, F) in fiber tracts of the intermediate zone (IZ) ([E, G], asterisks), subplate (SP), radial fibers in the cortical plate (CP), and marginal zone (MZ). At higher magnification, PrPC-IR (F) parallels GAP-43 (G) and synaptophysin labeling (H). Note the presence of PrPC-positive cell bodies in the subplate (F). Scale bars = (A, C, E) 5 mm. Original magnification: (F, G, H) 100×.
Controls

Negative controls were performed by omitting the primary antibodies or by using nonspecific isotype-matched primary antibody or irrelevant antiisotype secondary antibody.

RESULTS

PrPC immunostaining was observed in axonal fiber tracts and various cell types in the developing telencephalon (Table 3). Among the 5 antibodies used in this study, 12F10 and 6H4 produced similar, strong, specific reactions with PrPC, whereas BG4 produced weaker fiber tract staining compared with the other 4 antibodies. Under our working conditions, KG9 and 3F4 antibodies produced additional widespread nuclear membrane and chromatin staining with both the peroxidase and the immunofluorescence techniques. However, the overall density and intensity of PrPC-labeled cell bodies was similar with the 5 antibodies. Although labeling on cryostat sections was similar to that on paraffin sections, additional positive cell bodies, especially microglial cells, were detected on the paraffin sections in the early stages of gestation (virtual slides of PrPC immunohistochemistry in human telencephalon at midgestation using the 5 antibodies are available at: http://teleslide.crihan.fr/forum/pu_subjects.php?forumid=114).

11 to 23 Gestational Weeks

The main nuclei and large-fiber tracts in the telencephalon and diencephalon were individualized at these stages. The cerebral wall anlage showed the following layers: marginal zone (future layer 1), cortical plate, subplate, intermediate zone (future white matter), subventricular zone, and ventricular zone.

PrPC-Labeled Fiber Tracts

From 11 gw on, PrPC immunoreactivity was observed in the fiber tracts of the developing fornix at the septal level, internal capsule, cerebral peduncle, and penetrating the cortical plate (Fig. 1A). These fascicles were clearly visualized on contiguous GAP-43-labeled sections (Fig. 1B). Developing fascicles of slender fibers were visible between the nuclei of the caudate, putamen, and thalamus at 11 gw, and their staining intensity increased with age until 18 gw (Fig. 1C). Within the caudate and putamen, small fascicles of fibers were PrPC-immunoreactive. Subcortical PrPC-labeled tracts developed earlier than did the cortical axonal field. In the cortical anlage, PrPC-labeled axons were seen in the marginal zone, in the

FIGURE 2. PrPC (12F10) in the hippocampus. 18 gestational weeks (gw) (A), 23 gw (B), perinatal (C), and adult (D). The pattern of PrPC immunoreactivity (A1–D1) is very similar to that of GAP-43 (A2–D2) and synaptophysin labeling (A3–D3). (A) PrPC-immunoreactive axons are visible in the developing alveus (A) and in the thin band of stratum oriens (O). The dense labeling of the stratum radiatum and the stratum moleculare probably reflects the development of the perforant pathway (PP). No immunoreactivity is detected in the granule cells (G) of the dentate gyrus, whereas faint PrPC-IR, probably related to the mossy fibers, is visible near the CA3 field (asterisks). (B) Note the dense labeling of the molecular layer of the dentate gyrus (M). (C) In the perinatal period, PrPC-IR is intense and homogeneous in various ammonic fields. (D) In adulthood, PrPC-IR decreases markedly in the fiber tracts such as the alveus, fimbria, and stratum lacunosum–moleculare, which represent the perforant pathway. The stratum lacunosum–moleculare (L) is unlabeled on the GAP-43-labeled sections (D2). The granule cells remain unlabeled for PrPC, whereas the molecular layer of the dentate gyrus (M) remains labeled (D1). PrPC-IR increased in the neuropil. Scale bars = (A, B) 2 mm; (C, D) 5 mm.
subplate, running tangentially to the intermediate zone, and penetrating radially into the cortical anlage (Fig. 1F). A similar distribution was seen on contiguous sections labeled with GAP-43 and synaptophysin (Fig. 1F) and in the developing hippocampus (Fig. 2A, B) compared with perinatal (Fig. 2C) and adult (Fig. 2D).

**PrPC-Labeled Cell Bodies**

From 13 gw on, PrPC-positive cell bodies were detected around vessels in the ventricles and meningeal layers (and within the subplate [Fig. 1F] and germinal zones) and later in the cortical plate. Their density varied across cortical areas and they were detected chiefly in patches in the subcortical areas (Fig. 3). PrPC-Iba1 double labeling showed that almost all these cells were microglial–macrophage cells (Fig. 3A–D). PrPC was detected in developing microglia that exhibited a macrophage phenotype (ameboid microglia) (Fig. 3A–C) as well as in resident microglia (Fig. 3D). Few PrPC-labeled cells expressed MAP2 (Fig. 3E) or doublecortin (not shown) at this age in the cortical plate or subplate. No PrPC-GFAP double labeling was detected.

**25 to 32 Gestational Weeks**

PrPC labeling in axonal tracts and fascicles became stronger in parallel with the increased numbers of elongating axons within the nuclei, cortical anlage, and hippocampus. At the same time, granular synaptic-like PrPC immunoreactivity increased in the gray matter.

At 25 gw, the density of PrPC-positive cells diminished considerably in the paraffin and frozen sections. The microglial–macrophage cells, most notably those exhibiting Iba1 immunoreactivity in cryostat sections, lost most of their PrPC expression around 30 gw. In parallel, some of the PrPC-expressing cells were double labeled with calretinin and MAP2 in the superficial subplate and cortical plate. Some of these cells were distributed in patches.

**Perinatal Period (33 gw to birth)**

**PrPC-Labeled Axonal Field**

In low-power fields, PrPC immunoreactivity was more intense and of similar intensity (Fig. 4A) in the gray and white matter. At higher magnification, strong PrPC immunoreactivity

![Figure 3](http://jnen.oxfordjournals.org/...)

**Figure 3.** PrPC (12F10)-expressing cell types at midgestation. (A–D) Double labeling using PrPC and Iba1. All colors are combined in the righthand columns (A3–D3). (A) PrPC-Iba1 double labeling in paraffin sections displaying PrPC-labeled microglial cells in the subplate. Fine cell processes of ramified microglial cells ([A2], arrow) are not PrPC-labeled on paraffin sections. (B–D) PrPC-labeled microglial cells are more numerous on frozen sections than on paraffin sections. (B) Ameboid perivascular PrPC-labeled microglial cells (arrows) in the ventricle near the choroid plexus, and intermediate PrPC-labeled microglial cells within the brain parenchyma. (C) PrPC-labeled microglial cells forming a cluster in fiber tracts and (D) intermediate PrPC-labeled ramified microglial cells in the subplate. (E) 12F10-MAP2 double labeling reveals that few neurons express PrPC at midgestation. Scale bars = (A, B, E) 10 µm, (C, D) 20 µm.

![Figure 4](http://jnen.oxfordjournals.org/...)

**Figure 4.** PrPC pattern of immunoreactivity during the perinatal period. (A) Low magnification showing strong PrPC-IR in the white and gray matter in the perinatal period. Scale bar = 5 mm. (B) In the neocortex, PrPC immunoreactivity of neurons, predominantly in ischemic areas, and granular neuropil PrPC-IR that probably corresponds to synapses. Original magnification: 200×.
was seen in the axonal compartment of the white matter and in the neuropil of several nuclei and cortical layers (Fig. 4B). Similar GAP-43 immunoreactivity was observed in gray and white matter on contiguous sections of various regions, including the hippocampus (Fig. 2C1, C2). The pattern of synaptophysin labeling was similar to PrPC immunoreactivity (Fig. 2C1 compared with 2C3).

PrPC-Labeled Cell Bodies

In the perinatal period, patches of PrPC-labeled cell bodies were distributed in the cortical plate (Fig. 4B). Cell shape and double labeling properties indicated that these cells were neurons (Fig. 5A). Two of our specimens displayed sparse anoxic–ischemic lesions, and PrPC-immunoreactive neurons were located predominantly at sites of cortical ischemia. In contrast, only a few microglial cells present in these forebrain areas expressed PrPC immunoreactivity (Fig. 5B, C), mainly around vessels. A few PrPC-immunoreactive astrocyte end feet were seen along the vessel walls (Fig. 5D, E).

Adult

In contrast to findings in perinatal specimens, PrPC immunoreactivity was very weak in adult white matter compared with the cortex (Fig. 6). Its distribution was similar to that of GAP-43 and synaptophysin on contiguous sections. The immunoreactivity pattern in the hippocampus was similar to that described elsewhere (Fig. 2D) (6, 28). PrPC immunoreactivity was observed in a few perivascular macrophages.

PrPC Is Expressed in Vessels, Ependymal Cells, and Choroid Plexus

Dispersed ependymal cells (Fig. 7A, B) displayed PrPC immunoreactivity at all stages of gestation. PrPC immunoreactivity was not seen in ependymal cells in the single adult case. Choroid plexus was PrPC-labeled at all ages (Fig. 7C). PrPC immunoreactivity was seen occasionally in various vessel wall cell types, most notably endothelial cells (Fig. 7D) and in intravascular cells.

DISCUSSION

PrPC expression was found in fiber tracts and various cell types in human forebrain during development. PrPC expression in fiber tracts and fascicles was detectable at 11 gw. PrPC labeling was noted at birth both in axons and in neuronal perikarya. Patches of neurons expressed PrPC, most notably at sites with anoxic–ischemic changes as previously reported (16). Development of the PrPC axonal synaptic terminal field paralleled that of the synaptic protein synaptophysin and during adulthood, PrPC labeled the synaptic neuropil (6, 28). Interestingly, strong microglial PrPC expression was detectable from 13 gw until midgestation in migrating and differentiating cells. This microglial PrPC expression decreased toward the end of gestation, even in specimens exhibiting ischemic changes, particularly where the microglia was activated.

Antibody Comparison

Five antibodies were evaluated at all ages and, based on the results, 12F10 and 6H4 were selected. Overall, staining of fiber tracts and cell bodies was similar with the 2 antibodies. BG4 produced fainter staining of fiber tracts, which possibly indicated the presence of N-truncated isoforms of PrPC, as reported previously (29, 30). Alternative explanations included differences in antibody affinity or decreased epitope availability related to protein conformation or postmortem degradation. We did not seek to determine whether our specimens contained...
several PrPC isoforms, because the quality of the PrPC protein was probably affected by the conditions associated with abortion and by postmortem changes. In our study, 3F4 and KG9 produced widespread nuclear staining. The intracellular trafficking and the metabolism of PrPC have not been fully elucidated (31) and location of the protein is still a matter of debate (4–7, 32). However, nuclear staining by 3F4 was reported by Rybner et al (33). In our study, the use of both paraffin and frozen sections and the short time from death to fixation support the reliability of our results.

**PrPC Is Expressed in Growing Axons and in Synapses Throughout Synaptogenesis**

Axonal PrPC immunoreactivity followed the gradient of cerebral development. In general, axonal fields develop earlier in the diencephalon and the ventral telencephalon than in the cortex. Fiber tract PrPC immunoreactivity was readily detectable at 11 gw, suggesting earlier expression in humans than in rodents (14, 15). A remarkable feature of early PrPC expression in fiber tracts in the cortical anlage was its concentration in the subplate, just beneath the cortical plate, as well as in the marginal zone. Interestingly, we detected an accumulation of dopaminergic and noradrenergic afferents toward the human cortical anlage around 12 gw in these same locations (25). Later, these afferents penetrated the cortical plate, as observed for PrPC-labeled fibers. These PrPC-immunoreactive fiber tracts were probably developing axons, because they colocalized with GAP-43 (34), which is among the most abundant proteins in neuronal growth cones (35). These data support in vitro findings showing expression of prion protein in beta-tubulin-positive axons of embryonic retinal explants of hamster (14). Experimental data suggest PrPC involvement in neurite outgrowth (17, 21). In addition to growing axons, synapses expressed PrPC at increasing levels throughout synaptogenesis (36, 37). High levels of PrPC are found in synapses in adults (7). We also found weak PrPC immunoreactivity in normal white matter in adults. Earlier studies have established that PrPC is transported along axons, increases during axon regeneration (38, 39), and parallels beta APP immunoreactivity (16).

**PrPC Is Expressed in Neurons**

PrPC-expressing neurons were few at midgestation and increased gradually after neuronal migration was completed. As described by McLennan et al, we detected clusters of PrPC-immunolabeled neurons in perinatal specimens, most notably at ischemic sites (16). McLennan et al (16) showed that cerebral infarct size in PrP-null mice was significantly greater than in wild-type mice, supporting a role for PrPC in the neuroprotective cellular response to hypoxic injury. PrPC has been shown to play a role in the regulation of oxidative stress (23) and may influence the control of neuronal survival (20–22).

**PrPC Is Expressed in Microglial Cells**

Microglial cells and macrophages express PrPC mRNA and proteins (2, 9, 40, 41). In our study, until midgestation, the most numerous PrPC-immunoreactive cells were amoeboïd and intermediate microglial cells representing migrating and differentiating microglial cells (42, 43). They were found chiefly in fiber tracts, invading the gray matter and differentiating into resident ramified microglia. Interestingly, in contrast with neuronal expression of PrPC, microglial expression decreased gradually from midgestation onward, nearly disappearing by the perinatal period. PrPC is abundant in immune cells (44–46) and has been detected in perivascular
brain macrophages more frequently than in parenchymal microglial cells in the adult human brain (41).

Our data suggest that, during development, PrPC expression by microglial cells may be unrelated to microglial activation as ascertained by HLA-DR expression. The ischemic perinatal brain showed almost no PrPC in reactive microglia. Interestingly, HLA-DR is expressed in only a small number of cells before midgestation ([42, 43] and unpublished observation by CV), whereas it is present in larger numbers of cells during the perinatal period when microglial PrPC expression is significantly reduced. In a large series of adult cases, no correlation was found between HLA-DR expression and microglia/macrophage PrPC expression (41).

Thus, PrPC may play a role in the early differentiation stages of microglial subpopulations. Prion protein activates various signaling cascades in macrophages (47) and modulates phagocytosis (48). However, the functions of microglial cells during gestation are not clearly understood (49, 50).

### PrPC Is Expressed in Other Nonneuronal Cells

We found PrPC immunolabeling of choroid plexus at all ages, including adulthood. Ependymal cells were also labeled throughout gestation. Similarly, PrPC mRNA expression in the choroid plexus has been found at various developmental stages in animals (2, 14) and in ependymal cells of adult humans (3). PrPC immunoeexpression was detected in perivascular astrocytes in late gestation. Astrocytes express PrPC mRNA in neonatal and adult rodents (2, 51). Astrocytic PrPC expression has been shown to play a role in the regulation of copper and glutamate uptake (52, 53). We noted vascular PrPC immunoeexpression in abnormal adult and fetal specimens excluded from this study on the normal developing brain. We detected PrPC immunolabeling of various cell types in vessel walls of normal brains, which have been shown to express PrPC mRNA and protein (2, 54).

In summary, the distribution of PrPC axonal and cellular staining changed during development. Thus, PrPC expression seems to be regulated in neuronal compartment and in microglial cells on developing brain. Further studies are needed to elucidate the functions of PrPC in specific cell types in normal and abnormal brain.

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