Astrocytes in transmissible spongiform encephalopathies (prion diseases)

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Astrocytosis is one of the hallmarks of neuropathology of transmissible spongiform encephalopathies (TSEs) or prion diseases. In this review, we summarize data on astrocytic reaction, including naturally occurring or experimentally induced TSEs. A particular form of astrocytic reaction is known as gliosis and it is typical for experimental scrapie in mouse or in hamsters. Also, astrocytes participate in the formation of amyloid plaques. An interesting interaction between astrocytes and oligodendrocytes is discussed in detail as well as a particular form of astrocytic reaction in panencephalopathic form of TSEs.

key words: prion diseases, astrocytes, gliosis, PrP

INTRODUCTION

Astrocytosis, or reactive gliosis, is a prominent feature of naturally occurring and experimentally produced transmissible spongiform encephalopathies (TSEs) [83, 86, 87]. In this chapter we shall review diverse aspects of astrocytic gliosis in naturally occurring and experimentally induced TSEs.

KURU

Natural disease

Historically, emphasis has been placed on astrocytic hypertrophy and proliferation as the hallmark of kuru [64, 65] and this has been supported by more recent systematic immunohistochemical studies [49] in which astrocytic proliferation was found to be widespread and more abundant in the grey matter than in the white. Astrocytosis paralleled neuronal depopulation, although it has also been observed in regions with only minimal brain pathology. In the pons, severe gliosis was observed in the tegmental and basal portions with a conspicuous paucity of pyramidal tracts and medial lemnisci. Gliosis was severe in the midbrain, basal ganglia, thalamus, subcortical white matter and in the cerebellum, where it was mainly the vermis that was affected. Fagnans’ cell proliferation was conspicuous. In the cerebral cortex the proliferation of astrocytes was in excess of other pathological changes. Astrocytosis was diffusely present in the anterior horns of the spinal cord. Some astrocytes showed clasmatodendrosis. Furthermore, Neuman et al. [104] observed severe astrocytosis in three kuru patients, while Scrimgeour at al. [118] found only mild astrocytic changes with the presence of rare bi-nucleated forms in the cerebral cortex.

Experimental studies

Experimental kuru in chimpanzees is characterised by widespread astrocytosis and both hypertrophy and proliferation of astrocytes have been observed [3, 4, 69]. Gliosis seemed to parallel the severity of spongiform change and neuronal loss, being most abundant in the markedly vacuolated sensory cortex and less so in better preserved motor areas. Striatum, diencephalon, the white matter and the cerebellum showed severe glio-
sis. Ultrastructurally, astrocytes showed focal and, in our opinion, artefactual clearings of the cytoplasm and accumulations of glycogen granules [69]. In a separate unpublished study of early changes in New World monkeys infected with kuru Liberski, Brown and Gajdusek found, using GFAP-immunohistochemistry and electron microscopy, only moderate astrocytosis. Interestingly, astrocytes were observed adjacent to cerebellar granule cells undergoing faulty myelination. The biological significance of this phenomenon is unknown.

CREUTZFELDT-JAKOB DISEASE (CJD) AND GERSTMANN-STRÜSSLER-SCHEINKER DISEASE (GSS)

CJD

Natural disease

Varially severe reactive astrocytosis is observed among almost all neurodegenerative conditions and CJD is no exception. Hypertrophic astrocytes, detected by means of metal techniques (the Holzer, Kanzler or Cajal methods) (Fig. 1) or currently by immunostaining against glial fibrillary acid protein (GFAP), are seen in all vacuolated areas (Fig. 2). In the cerebral cortex they are particularly prominent in the deeper cortical layers. Large gemistocytic forms are frequently observed. When destruction is so severe that it leads to the collapse of vacuolated neuropil, proliferating astrocytes may virtually replace all other cellular elements. In such a situation the spongiform changes may no longer be recogn-

Figure 1. Reactive astrocytes in an autopsy case of Creutzfeldt-Jakob disease. Cajal gold sublimate. Courtesy of Prof. Herbert Budka, Medical University, Vienna, Austria.

Figure 2. Reactive astrocytes in an autopsy case of Creutzfeldt-Jakob disease. GFAP-immunohistochemistry.
192.76 ± 117.98 per mm² in controls to 278.08 ± 137.73 per mm² in CJD. An increase in the cross-sectioned nuclear area of Bergmann glia (32.72 ± 6.8 μm² vs 42.75 ± 9.61 μm²) and of velate astrocytes (34.86 ± 7.29 μm² vs. 39.37 ± 7.10 μm²) was seen when control values were compared with those of CJD. It is noteworthy that the basic three-dimensional geometry of the astrocytic scaffold of the cerebellum was maintained despite severe loss of granule cells. Electron microscopy revealed several subcellular organelles, rare but otherwise typical for reactive astrocytes, single cilia consisting of ciliary shafts (Fig. 4), clusters of interchromatin and perichromatin granules, various adhesive plaque junctions (Fig. 5) and simple and granular nuclear bodies. Of particular interest is the presence of infoldings of the plasma membranes in the perivascular regions of the astrocytic end-feet. These infoldings were covered by an interrupted or continuous electron-dense undercoat of 30–60 nm in diameter. The latter observation is in agreement with the earlier freeze-etching study of Dubois-Dalcq et al. [32], who showed an increase rather than a depletion in the number of astrocyte-specific particles on membranes forming vacuoles.

**Experimental studies**

Astrocytosis presents a substantial part of the neuropathological picture of experimental CJD. In the first reported transmission experiment Beck et al. [5] found moderate to severe astrocytosis in both biopsy and necropsy specimens of CJD virus-infected chimpanzees (Fig. 6). In the cerebral cortex the hypertrophic astrocytes completely distorted the neuronal architecture. Many astrocytes were of the gemistocytic type, similar to those in human CJD. Severe glial reaction was also seen in the striatum, diencephalon and cerebellar cortex. Beck et al. [5] raised the problem of astrocytes as a primary target for the CJD agent, in other words the location of CJD within a vague spectrum of so-called “glial dystrophies”. This notion was based primarily on observed discrepancies between the severity of astrocytosis and neuronal damage. While such differences have been unequivocally noted, it must be stressed that in most situations the most severely vacuolated brain regions also presented the highest level of astrocytosis. Manuelidis et al [62, 63, 95, 96] found particularly severe astrocytosis in experimental CJD in guinea pigs, hamsters and mice. In CJD-affected hamsters clusters of these cells appear almost as pure astrocytic cultures. This collection is far in excess of what is traditionally known in human and experimental neuropathology as “reactive astrocytosis”. In order to further substantiate the notion of a primary involvement of astrocytes in CJD, Manuelidis and Manuelidis reported that astrocytes from CJD-affected brains could be maintained in vivo (immortalised) for a long period [96]. In contrast, those established from uninfected brains all died after a short time. This problem was further addressed in a “serial killing” experiment, in which we found by means of electron microscopy that astrocytosis paralleled spongiform change in the parietal cortex and adjacent corpus callosum of mice infected with the Fujisaki strain of the CJD agent [90]. As dilated and swollen astrocytic processes were found occasionally in both CJD-infected and sham-inoculated animals, these were regarded as a result of local suboptimal fixation and not, as reported by others [62, 63, 77, 78], as part of the CJD neuropathology.

It is of note that certain aspects of the neuropathology of TSEs have been partially reproduced in transgenic mice, created by microinjection of the chimeric murine cosmid containing a codon 101 Pro to Leu substitution in the ORF of the mouse PrP gene [52]. The 101 codon substitution is regarded as an equivalent to that found

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**Figure 3.** Type IV according to the classification of “nuclear bodies” of Bouteille et al. [11].

**Figure 4.** A reactive astrocyte containing single cilia consisting of ciliary shafts.
in GSS (there is a deletion of codon 55 in the mouse PrP gene) [50, 51]. Transgenic mice presented severe spongiform change but somewhat mild or moderate astrocytosis except in the cerebellum, where severe Bergmann radial gliosis was observed.

**GSS (see also Gerstmann-Sträussler-Scheinker disease, this volume)**

While astrocytic gliosis is a prominent and ubiquitous finding in CJD, kuru, scrapie and bovine spongiform encephalopathy, it still remains a rather controversial issue with regard to GSS (Fig. 7). Hudson et al. [53] found mild astrocytosis associated mostly with amyloid plaque in three cases of GSS. In a case reported by Kuzuhara et al. [66] moderate astrocytosis of the cerebellar white matter was found, while a severe astrocytic reaction was seen in the inferior colliculus. Vinters et al. [124] reported astrocytic gliosis throughout the neocortex, while Tateishi et al. [119], in contrast, found astrocytosis only in an area of concomitant infarct. Similarly, Ghetti et al. [40], Nochlin et al. [105] and Pearlman et al. [108] reported severe gliosis only in areas where numerous plaques and neuronal loss were also seen. In three cases of GSS studied by us at the Laboratory of Central Nervous System Studies (LCNSS), National Institutes of Health in Bethesda and in the Neurological Institute of the Medical University of Vienna astrocytosis was found throughout the cerebral and cer-

**Figure 5A–E.** Different forms of intercellular junction.
Figure 6. The neuropathology of experimental Creutzfeldt-Jakob disease in a chimpanzee. Courtesy of the late Dr. C.J. Gibbs Jr, Laboratory of CND Studies, NINDS, NIH, Bethesda, MD, USA.
ebellar cortex, although the severity of this change never approached that found in CJD cases. In particular, gemistocytic astrocytes were never seen in these cases but astrocytes were characteristically elongated and slender, reminiscent of pilocytic astrocytes. However, in a recent case from the original Austrian GSS family, astrocytosis in the cerebral cortex approached that of CJD brains and innumerable gemistocytic astrocytes were seen [48, 79, 84]. Thus the diversity of the neuropathology of GSS is perhaps of the same magnitude as that of CJD.

THE INVOLVEMENT OF ASTROCYTES IN THE FORMATION OF AMYLOID PLAQUES

Amyloid plaques are a neuropathological feature of TSEs [54]. There is an “unnecessarily complex” [54] classification of amyloid deposits in humans into several partially overlapping categories [125]. Kuru plaques and multi-centric plaques are characteristic features of kuru (or CJD) and GSS, respectively. Cortical kuru (unicentric) plaques of GSS consist of amyloid fibrils within a narrow extracellular space between distended astrocytic processes (Fig. 8). Amyloid fibrils invaginated “deeply the surrounding profiles of astrocytes so that the filaments sometimes seemed to be intracellular” [8–10]. Such peripheral accumulations of astrocytic processes in close proximity to the amyloid fibrils were noted even in the earliest amyloid plaques. This intimate association of amyloid and astrocytes in GSS led Boellaard et al. [9] to coin the term “glial plaques”. Glial plaques are plaques of TSEs and contrast with the neuritic plaques of Alzheimer’s disease [136], although, like the latter, they are invaded by microglial cells [2, 102]. A systematic immunohistochemical approach disclosed that 30–50% of unicentric plaques contain microglia cells, while astrocytes are located around these plaques with long processes penetrating them [102]. In contrast, only a proportion of multi-centric plaques contain microglial cells, although the pattern of astrocytic involvement is virtually the same. These authors also distinguish “cores with satellite deposits” (a variant of multi-centric plaques). Of the latter 70–80% contain microglia cells; the pattern of astrocytes remains unchanged.

In contrast, diffuse (primitive) plaques in mice exhibit neither amyloid cores nor amyloid filaments [55–58]. They are infiltrated neither by astrocytes nor by microglial cells. However, as plaques mature and the PrP within them fibrilises, the number of both microglial cells and astrocytes tends to increase. Thus it seems that both categories of glial cell may be merely reactive. The elegant immunogold studies have shown that PrP is indeed localised to the lysosomal compartment of these cells.

SCRAPIE, BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) AND CHRONIC WASTING DISEASE (CWD)

Scrapie

Natural disease

Generally, the degenerative brain pathology of natural sheep and goat scrapie consists of spongiform change and astrocytosis. The latter change is highly variable (Fig. 9); many cases of natural scrapie in sheep show inconspicuous or undetectable astrocytosis. In the majority of textbook descriptions “neuronal loss” is also mentioned. However, the only reference supporting this
statement is that of Beck et al. [6], who studied those brain areas that are characterised by highly variable numbers of neurons. In contrast, neuronal loss does occur in BSE [59].

The true nature of astrocytic change is only poorly understood and one of the most pertinent problems in studying glial responses in scrapie is still whether astrocytic proliferation (hyperplasia) or only astrocytic hypertrophy or both are responsible for the apparent increase in the number of astrocytes observed under the light microscope [37].

Hadlow [46], studying scrapie-infected dairy goats, reported that both hypertrophy and hyperplasia, the former predominating, brought about an overall increase in the apparent number of astrocytes seen in sections. Estimation of the number of astrocytes was not easy, however, as the Cajal method also involved staining different proportions of astrocytes in normal goat brains. The “scrapie” astrocytes were not always easily distinguished from pleomorphic “normal” astrocytes but typically they measured up to 14 μm in diameter and contained a few chromatin granules. Kidney-shaped, elongated and irregularly lobulated nuclei have been reported in particular, frequently clustered as 3 or 4 cells reminiscent of those in Alzheimer II cells or the “naked nuclei” of Alzheimer. The hypertrophy and proliferation of astrocytes were confined to the affected (vacuolated) grey matter. The adjacent white matter was involved only occasionally. However, in the midbrain and several thalamic nuclei astrocytosis predominated over vacuolation, while the cerebral cortex, characterised by minimal spongiform change, presented, occasionally disproportionately, spectacular astrocytosis.

Topographically, different brain regions were involved to a different degree. The lesions were bilaterally symmetrical and the boundaries between affected and unaffected regions were remarkably sharp. Dense astrocytosis was observed in the pallidum, septal nuclei, and diencephalon. Moderate astrocytosis was seen in the striatum and the brain stem where hypertrophy prevailed above hyperplasia. Minimum astrocytic hypertrophy with slight proliferation was seen in the deeper layers of the otherwise mostly unaffected cerebral and cerebellar cortices. In these areas radially arranged glial processes and clusters of Bergmann glia were the distinctive features. The hippocampus formation was unaffected but diffuse astrocytosis was evident, mostly between the pyramidal cells and the alveus. Hypertrophy and “undoubted” proliferation have been also detected in natural scrapie in goats [47]. Topographically, astrocytosis of both experimental and natural caprine scrapie were alike, except that the striatum, pallidum and septal nuclei were only slightly affected in the latter. A similar increase in the number of hypertrophic astrocytes was noticed in sheep with natural scrapie [39]. It was of note that the number of astrocytes diminished with age both in controls and in scrapie-affected sheep. In the latter group, however, this decrease was not as pronounced. No association was found between the degree of astrocytosis and the duration of the clinical disease or with the severity of spongiform change.

Experimental studies

Pattison and Jones found that hypertrophy but not proliferation was a feature of rats infected with the Chandler strain of scrapie agent [106]. Astrocytosis mostly paralleled spongiform change and was greater after intracerebral inoculation than after intraperitoneal inoculation. Astrocytosis preceded the vacuolation by 14 days. Astrocytic end-plates were hypertrophic, and in the later stages of disease “capillaries appeared to be embedded in swollen, darkly staining astrocytic cytoplasm”.

The problem of hypertrophy versus hyperplasia has been studied by Hugh Fraser and colleagues [38, 117] in several models of murine scrapie, and by Liberski and colleagues [73–75, 81] in hamsters infected with the 263K strain of scrapie agent. Fraser [38] coined the term “gliocytosis” to denote the proliferation of astrocytes accompanied by changes in their morphology and substantial proliferation of rod-like microglial cells. In murine scrapie gliocytosis, encountered in the hippocampus and the thalamus, is an extremely rare phenomenon found in approximately 3% of 10,000 murine scrapie-affected brains. Gliocytosis occurs in a wide range of scrapie isolates passaged in different strains of...
mice but almost exclusively after intracerebral inoculation (256 examples of 260 studied brains with gliocyto-
sis) [38]. In more detailed studies of gliocytosis (sclero-
sis) of the hippocampus formation Scott and Fraser [117] found its presence paralleled that of severe vacuolation.

Liberski and colleagues found gliocytosis in hamsters infected with the 263K strain of scrapie in a much higher proportion than in the murine scrapie models [73–75, 81] (Fig. 10). Both astrocytic hypertrophy and proliferation were observed. Astrocytosis apparently correlated with spongiform change but not with neuronal loss. In the hippocampus astrocytic changes were seen in both the pyramidal cell layer and the granular cell layer of the fascia dentata. Astrocytic hyperplasia was evident and different stages of mitoses were recognised (Fig. 10D). Many astrocytes were similar to the “naked nuclei” of Alzheimer II cells (Fig. 10C), others contained lobulated and bizarre nuclei more reminiscent of those of hyper-
trophic reactive astrocytes (Fig. 10E–F) or astrocytes en-
countered in multi-focal leukoencephalopathy. The pres-
ence of glial fibres and Rosenthal fibres, regarded as prod-
ucts of glioflament condensation and degeneration, were frequently noted. The proliferation of astrocytes was ac-
companied by rod-like microglial cells.

In order to clarify the proliferative potential of astro-
cytes in TSEs, we have studied immunohistochemically the immunoreactivity of proliferating cell nuclear anti-
gen (PCNA) [7], an auxiliary protein of polymerase δ which is active in DNA leading-strand synthesis and then an es-

Millerski et al. found gliocytosis in hamsters 9 weeks after intracerebral inoculation, while at that time astrocytosis unequivo-
cally surpassed vacuolation. Masters et al. [100] found no PCNA expression was observed in the brains of con-
rol animals.

In CJD-affected mouse brains PCNA LI correlated significantly with the grade of astrocytosis in the deep
layers of both the cerebral cortex and the corpus callo-
sum (r = 0.78 and 0.5; p < 0.01 and < 0.05, respective-
ly) but not in the subependymal zone or the cerebellar
white matter. The correlation of PCNA LI and incubation time (measured in weeks) was statistically significant
only in the subependymal zone (r = 0.41; p < 0.05), while
the grade of astrocytosis correlates significantly with the
incubation period only in the deep layers of the cere-
bral cortex and in the subependymal zone (r = 0.47 and
0.51; p < 0.05 and 0.01, respectively).

In CJD-affected mice at all stages the number of
PCNA-immunopositive astrocytes was low, less than 5%
of the visible population of astrocytes (the highest PCNA
LI, 4.5%). By contrast, in brain tissues from human pa-
tients with kuru, CJD and GSS in which abundant PrP-
immunopositive plaques were seen [2, 44] no PCNA-
immunopositive cells were detected despite the pres-
ence of numerous microglial cells and reactive astro-
cytes, which were clearly identified on adjacent sections
such as those following immunostaining with antibod-
ies against ferritin and glial fibrillary acidic protein (GFAP)
respectively.

Ultrastructural studies are in general agreement concerning glial changes [19–21]. Astrocytes showed
no features distinguishing them from the reactive as-
trocytes found in a plethora of neurodegenerative dis-
orders (Fig. 10).

The few “serial killing” experiments performed so far provide conflicting data on whether astrocytosis ap-
pears before or after vacuolation. Marsh and Kimber-
lin [99] found hypertrophic astrocytes in scrapie-infected
hamsters 9 weeks after intracerebral inoculation and
preceding vacuolation by 2 weeks. This initial
astrocytic hypertrophy was first observed at the pia-
arachnoid surfaces and adjacent to the ventricles. In
contrast, Liberski and Alwasiak demonstrated that
astrocytosis actually followed vacuolation in hamsters
infected with the 263K strain of scrapie agent [80].
Scrapie-specific vacuoles appeared 8 weeks following
inoculation, while at that time astrocytosis unequivo-
cally surpassed vacuolation. Masters et al. [100] found
astrocytosis detectable at weeks 7 or 5 by means of
routine neuropathological staining or indirect immu-
ofluorescence. Unequivocal spongiform change ap-
peared in this model 7–8 weeks after inoculation.
Figure 10. Experimental scrapie in hamsters (scrapie strain 263K). A. A typical reactive astrocyte; B. Reactive astrocytes in the vicinity of the vessel; C. Alzheimer type II cells (“naked nuclei”), haematoxylin and eosin; D. An astrocyte undergoing mitosis; E. Severe gliocytosis, haematoxylin and eosin; F. Dividing Alzheimer type II cell, haematoxylin and eosin; G. Part of the cytoplasm of an astrocyte; H. Numerous gliofilaments in the cytoplasm of an astrocyte.
While the spongiform change stabilised in intensity at weeks 9–10 following inoculation, the number of astrocytes increased steadily until the clinical phase of the disease and thus paralleled the steady increase in the infectivity titer. This correlation may suggest astrocytes as a target for agent replication and not merely as passively reactive cells. Unfortunately, both experimental studies suffered from the obvious weakness of the use of “poorly vacuolated” models. Thus the question of whether astrocytosis is merely a reaction toward the destruction of neuronal elements or whether astrocytes undergo primary proliferation and hypertrophy cannot be settled.

Scrapie passaged to cattle produced prominent but moderate astrocytosis and only minimal or no spongiform change in numerous brain structures [22, 24]. The latter distinguishes scrapie in cattle from BSE in the same species. Astrocytes cluster frequently. The topographic distribution of astrocytosis was reminiscent of sheep scrapie with septal nuclei prominently affected while the other forebrain structures were not. The other affected structures included the thalamus, midbrain tegmentum, especially the periaqueductal grey matter, the pontine nuclei and the nucleus of the solitary tract. In the cerebellum Bergmann glia increased in number. The moderate degree of astrocytosis was confirmed by GFAP-immunohistochemistry. Analogous findings were reported for cattle infected with TME [115].

**BSE AND CWD**

The data on astrocytic reaction in bovine spongiform encephalopathy (BSE) and chronic wasting disease (CWD) are very limited. In the first report of BSE by Wells et al. [126] only mild gliosis was noted in BSE-affected cattle brains and this was repeatedly confirmed [127, 128]. Liberski et al. [76, 93] found that numerous hypertrophic astrocytes, not infrequently bi-nucleated and containing abundant glial filaments, accompanied the neuronal degeneration. Jeffrey et al. [59] reported on astrocytosis in BSE-infected mice but, as astrocytic response is highly variable between different experimental models, this study has little relevance to the problem of astrocytosis in natural BSE. In a captive puma (Felis concolor) infected with BSE both astrogliosis and microgliosis were readily apparent; the latter formed typical microglial nodules [135].

Analogously, in chronic wasting disease (CWD) in mule deer, hybrids of mule deer and white tailed deer and Rocky Mountain elk, numerous hypertrophic astrocytes have been noticed [43, 45, 132–134].

**INTERACTION BETWEEN ASTROCYTES AND OLIGODENDROCYTES**

Interactions between astrocytes and oligodendrocytes [82] have previously been reported only in early lesions of multiple sclerosis (MS) and a few other conditions [110, 137]. Their appearance in both naturally occurring and experimentally induced TSEs suggests an early cellular event that may trigger further tissue destruction. In the brain biopsy of a patient with CJD [89] low-power electron microscopy revealed numerous examples of astrocytes and oligodendroglial cells in close juxtaposition to cellular membranes of one cell moulded on those of another (Fig. 11). Occasionally two oligodendroglial cells were seen in close contact with the same astrocyte. At higher magnification it was seen that both types of cell were connected by rare adhesive plaque junctions. These subcellular organelles were composed of two symmetrical or asymmetrical subplasmalemmal densities (attachment plaques) collectively forming “attenuated desmosomes” or “desmosome-like” structures. In both the 263K and 22C-H hamster models similar phenomena were observed. A narrow intercellular space between these attachment plaques was still visible but one or two intermediate lines were detectable. More complex structures were also seen in both hamster models. The astrocytic cytoplasm was penetrated by a few oligodendroglial processes or the oligodendroglial cells were completely surrounded by astrocytic processes, which formed multi-layered onion-like “collars” around these cells. Such interactions were previously reported in early lesions of MS [110] and at that time they were regarded as unusual and possibly specific for this demyelinating process. In a subsequent detailed study, however, Wu and Raine [137] showed that such interactions, while frequently encountered in MS lesions, are non-specific, being observed...
in some other brain disorders, including Krabbe’s disease, toxoplasma encephalitis and brain infarcts. The common denominator in all these processes is the presence of inflammatory lymphocytic infiltrates, which are otherwise minimal or totally absent in TSEs [103, 130]. It is of further interest that astrocytes and oligodendrocytes show weak electrical coupling in vitro, which has been interpreted as showing that these cells are physically connected [23, 111].

The significance of interactions between astrocytes and oligodendrocytes is unclear at the present time. As in MS and other brain lesions in which it has been studied, this interaction is not associated with a response to any infectious pathogen. Rather it may be an event that triggers brain tissue destruction, which is eventually mediated by pro-inflammatory cytokines secreted from astrocytes, lymphocytes and macrophages.

A PARTICULAR FORM OF ASTROCYTIC REACTION IN TSES

The majority of TSEs are polioencephalopathies (diseases of the grey matter) and the corresponding fine structural changes are relatively well described. However, the panencephalopathic type of GSS, characterised by the predominant involvement of the white matter, has also been reported [120] and axonal and myelin pathology at the ultrastructural level has been described [85, 90–92].

Myelinated axons presented various pathological lesions. These changes were observed simultaneously in different areas of the same sample but the following description is organised as if it followed a sequence of events. Initially the myelin sheath was separated by cytoplasmic tongues into several concentric bands (Fig. 12). Cellular processes penetrated between layers of myelin and lifted away the outermost lamellae (Fig. 13). A complicated labyrinth of concentric cellular processes, clearly belonging to either the astrocytes or macrophages, invested the myelinated axons (Fig. 14). In the terminal stages axons completely denuded of myelin were seen in the centre of a concentric network of cellular processes or spirals of myelin were seen surrounded by such processes (Fig. 15). The myelin fragments penetrated the astrocytes or macrophages, where they underwent final digestion (Fig. 16). Macrophages containing fragments of axons, paracrystalline lamellar bodies and myelin debris were abundant in this model.

EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) AND ITS MRNA

First isolated from mature multiple sclerosis plaques [35, 36], glial fibrillary acidic protein (GFAP) is a major protein component of glial filaments, a class of intermediate filament specific to astrocytes. GFAP is a 49-kDa protein accompanied on polyacrylamide gel electrophoresis (PAGE) by products of a proteolytic cleavage of apparent molecular weight as low as 40 kD. It is regarded as a useful marker for normal, hypertrophic and neoplastic astrocytes.

Mackenzie studied Compton mice infected with the “Chandler” (139A) strain of scrapie by means of GFAP immunohistochemistry [94]. The use of GFAP as an astrocytic marker proved to be extremely useful, particularly for the quantitative estimation of astrocytosis, previously complicated by the insensitivity of routine haematoyxlin and eosin staining [73, 74] and the capriciousness of Cajal metal impregnation [38]. Abundant GFAP-immunopositive astrocytes were seen in the corpus callosum, hippocampus, cerebellum and spinal cord. This location was disease-specific, as a different pattern of GFAP im-
munoreactivity was observed in mice infected with Semliki forest virus or mice intoxicated with cuprizone. Furthermore, GFAP-immunoreactive astrocytes were readily detected in scrapie-affected sheep. It is noteworthy that there was no correlation between the clinical signs of scrapie and GFAP immunoreactivity in the brain stem, or between the distribution of spongiform change and GFAP immunoreactivity. The overproduction of GFAP was confirmed in mice infected with the C506 strain of the scrapie virus and in scrapie-affected sheep [1, 31, 71, 72]. Furthermore, GFAP mRNA paralleled the GFAP increase in the natural scrapie of sheep [1].

This astrocytic reaction, characterised by robust GFAP immunostaining, was referred to as “hypergliotic” [31], as it was out of proportion to the degree of neuronal damage. Furthermore, the regional distribution of GFAP-immunoreactive astrocytes paralleled that of PrP [25–27]. GFAP concentration, measured in homogenates of whole scrapie-affected hamster brain, increased 20–30 days following intracerebral inoculation [25]. The initial rise was slow and accelerated some 60 days following inoculation, when the first signs of clinical scrapie were also observed. PrP 27–30 was first detectable approximately 45 days following inoculation; thus the accelerated increase of GFAP concentration clearly followed that of PrP. A similar rise in PrP followed by an increase in GFAP was observed in selected brain regions. For instance, in the thalamus GFAP concentration increased 50 to 55 days after inoculation, while 5–10 days prior to this there had been an increased concentration of PrP. It thus seemed that PrP induced reactive gliosis. In order to test this hypothesis directly, the influence of PrP on astrocytic growth in vitro has been studied [25]. Primary astrocytic cultures (more than 90% astrocytes) exhibited a numerical increase in astrocytes on the third day on which PrP was present. Furthermore, a dramatic increase in GFAP-immunopositive glial filaments was observed following PrP supplementation of the culture medium. Such an increase in GFAP concentration paralleled that of an increase in GFAP mRNA. In conclusion, PrP was found to be a potent stimulant for astrocytes. It has been suggested that PrP released into extracellular spaces induces reactive astrocytic gliosis. PrP has also been localised to astrocytes [16]. In serial experiments PrP was detected in astrocytes 8 weeks following inoculation; it then increased to the stage at which it was detectable diffusely within the neuropil. The accumulation of PrP within the astrocytes preceded astrocytosis, first observed 12 weeks following inoculation, and the appearance of scrapie amyloid, seen 16 weeks after inoculation. From

Figure 14. Experimental GSS (the Fujisaki strain) in mice. A, B. A complicated labyrinth of concentric cellular processes, clearly belonging to either astrocytes or macrophages, invested the myelinated axons.

Figure 15. Experimental GSS (the Fujisaki strain) in mice. In the terminal stages axons completely denuded of myelin were seen in the centre of a concentric network of cellular processes or spirals of myelin were seen surrounded by such processes.
Figure 16. Experimental GSS (the Fujisaki strain) in mice. A–E. The myelin fragments penetrated the astrocytes or macrophages, where they underwent final digestion
the above discussion it seems that PrP, GFAP and astrocytosis are somehow related and that PrP is the growth factor for astrocytes.

Molecular studies of GFAP have taken an interesting turn. Wietgrefe et al. [129] constructed a cDNA from purified poly(A)RNA from scrapie-infected mouse brain. For differential hybridisation this cDNA library was screened by 32P-labelled cDNA reverse-transcribed from poly(A)RNA of scrapie-infected and control brains. One clone (Scr-1) hybridised preferentially to scrapie-infected brains. However, in dot-blot experiments Scr-1 was also shown to hybridise to control material, although to an extent that was 20-fold less. On northern blots Scr-1 hybridised to the 3.3 kb RNA species. In in situ hybridisation experiments Scr-1 was located to neurons, mostly in scrapie-affected brains, and to dystrophic neurites within neuritic plaques in human brains with Alzheimer’s disease and rare senile plaques of multi-infarct dementia brains [30]. While the significance of the Scr-1 gene was unknown at the time of its discovery, it has subsequently been established that the Scr-1 clone merely represented the 3’ non-coding region of GFAP [30, 97]. The Scr-1 cDNA sequence is 98% homologous to the 3’ untranslated region of the mouse GFAP cDNA. Indeed Scr-1 was further used as a probe to examine the expression of GFAP mRNA in CJD-infected hamsters [97]. The mRNA for GFAP was studied in regions that show no spongiform change and compared with those which exhibit severe vacuolation. An increased amount of GFAP mRNA was found in the cerebral cortex toward the end stage of the disease and its increase preceded the appearance of spongiform change. However, in some cerebral areas with prominent vacuolation its increase was not readily apparent. Conversely, a large increase in GFAP mRNA was noticed in the cerebellum, in which spongiform changes were absent. Analogous data were provided for scrapie-infected newborn mice [70].

Andreas-Barquin et al. [1] found upregulation at the protein and mRNA level of both GFAP and glutamine synthetase (GS). The latter finding might suggest that the traffic of glutamate and glutamine is distorted in scrapie but this was not confirmed in a subsequent study. GFAP is not, however, necessary for scrapie infection [41, 121]. Mice in which the first exon of the Gfap gene was disrupted by replacing it with lacZ gene (Gfap−/−) are susceptible to scrapie infection, develop typical pathology (including astrocytosis) and exhibit the same level and distribution (by histoblots) of PrP. Mutated astrocytes showed subtle differences in immunostaining with antibodies against vimentin and S-100 protein. Both vimentin and S-100 protein signals tend to be granular and limited in the perinuclear space, as opposed to wild type astrocytes, where both signals are rather filamentous and fill the whole cytoplasm.

It has, moreover, been demonstrated that not only GFAP but also several biologically active substances localised to astrocytes (metallothionein, crystallins, apolipoprotein E, cathepsin D and various lymphokines; see below) are upregulated in scrapie [18, 28, 29, 33, 61, 112, 131] and, further, that some of these compounds are also upregulated in another neurodegenerative disorder, Alzheimer’s disease, suggesting a convergent pathological mechanism [30, 41, 70]. Finally, tissue factor, the tissue activator of the coagulation cascade, was also upregulated in the astrocytes of scrapie-infected brains [34]. In experimental scrapie the upregulation of astrocytic enzymes precedes the development of neuropathological lesions but follows the rise in PrP [41]. Apolipoprotein E4, which is a risk factor for Alzheimer’s disease [116], may increase when astrocytes assume the role of macrophages, as demonstrated in experimental CJD [85, 90–92] and crystallin, being a heat-shock protein, may participate in the early response toward CNS damage [28, 29]. Collectively, these data suggest that astrocytes induced by PrP may play a substantially more important role in the pathogenesis of TSEs than merely reacting passively to other brain tissue lesions.

**ACKNOWLEDGEMENTS**


This paper has been supported in part by the Ministry of Science and Informatics L grant and by an Oead grant and is part of the EC NeuroPrion Network of Excel-lence.

**REFERENCES**


