Glial Inclusions in CNS Degenerative Diseases

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INTRODUCTION

There has been a renewed interest in glial pathology in central nervous system (CNS) degenerative diseases over the last few years. This is due to the availability of new methods that allow clearer visualization of glial inclusions, to a specific scrutiny of glial cells in such entities as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), corticobasal ganglionic degeneration (CBGD), Pick disease, and Alexander disease, and to recent definition of major molecular components of some of these inclusions. In this article, we will review the morphologic and biochemical characteristics of a variety of inclusions in astrocytes and oligodendrocytes in degenerative diseases, their distributions within the CNS, their specificities for cellular localization and for diseases, and discuss possible pathogenetic mechanisms and directions for future study. Many of these inclusions appear to reflect the impact of the disease on glial cells, rather than a secondary effect on glia due to neuronal degeneration. As such, the study of glial inclusions may lead to important insights into basic cellular mechanisms of disease.

GLIAL CYTOPLASMIC INCLUSIONS

The so-called glial cytoplasmic inclusion (GCI) is a well-recognized and specific histopathological finding of the nonfamilial forms of multiple system atrophy, including striatoni garal degeneration, olivopontocerebellar atrophy, and Shy-Drager syndrome. This pathologic entity was initially described by Papp et al in 1989 (1) and has subsequently been confirmed and further characterized by their group and a number of other investigators (2-4). On the light microscopic level GCIs are faintly staining, eosinophilic, glassy, intracytoplasmic inclusions that noticeably displace the glial nucleus into an eccentric position (Fig. 1a). These inclusions are argyrophilic and can be identified with silver impregnation stains such as the Bodian and modified Bielschowsky techniques. However, investigators have recommended additional modification of the latter technique to optimize staining of GCIs (2). The optimized procedures stain the inclusions black while the more routinely applied modified Bielschowsky silver stain results in a tannish-brown accentuation of the glial cytoplasm (Fig. 1b). It is probably because of this subtle staining that these inclusions have been overlooked in the past. The Gallyas silver technique and its various modifications (5-7) have become very useful stains for highlighting not only neurofibrillary tangle pathology in Alzheimer disease, but have been key stains in the identification and study of glial inclusions associated with MSA (1, 4) and other neurodegenerative disorders. The advantage of the Gallyas stain over the Bodian and modified Bielschowsky methods is its selective staining of the pathological inclusions without significant background staining of normal structures such as axons. GCIs do not stain with Klver-Barrera, Holzer, phosphotungstic acid hematoxylin, Mallory azan, alcian blue, nile blue, Masson trichrome, thioflavine S, oil red O, Sudan black B, periodic-acid Schiff, or Congo red stains (2, 3). The silver-impregnated inclusions demonstrate variation in shape and size. Most common are triangular, flame-shaped, crescent-shaped, and round to ovoid forms (Fig. 1c). The inclusions are localized within small- to medium-sized cells with nuclear features of oligodendrocytes and therefore have also been referred to as “oligodendroglial microtubular tangles” (2) or “oligodendroglial cytoplasmic inclusions” (8).

GCIs are composed ultrastructurally of a meshwork of randomly arranged, loosely packed filaments with cross-sectional diameters of 20 to 30 nm (average 25 nm) (1-3, 8, 9). The individual filaments have been noted to have a tubular appearance, although this has been questioned by some investigators. The filaments are coated for much of their length by electron dense granules or fuzzy material, which increases the effective cross-sectional diameter to approximately 40 to 50 nm. The filaments are cytoplasmic in location, entrapping organelles such as vesicles, mitochondria, and dense bodies; no limiting membrane surrounds the inclusions. Silver impregnation staining combined with electron microscopy have confirmed the argyrophilic nature of these structures (3). Structurally, these inclusions are very much similar to the neuronal cytoplasmic inclusions present in much lower numbers in the MSAs (10-13).
Fig. 1. Glial inclusions in neurodegenerative diseases. (a) GCIs appear as eosinophilic cytoplasm in oligodendrocytes. Cerebellar white matter. MSA. H & E stain. ×400. (b) GCIs can be detected with the modified Bielschowsky silver stain as brownish juxtanuclear bodies. Cerebellar white matter. MSA. ×310. (c) Gallyas silver stain dramatically highlights GCIs as densely black-stained material. Same area and case as in (a). ×350. (d) A tufted astrocyte in motor cortex is composed of a mass of thin, argyrophilic processes. PSP. Gallyas silver stain. ×400. (e) Tufted astrocytes are immunoreactive with antibodies against
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GCI's stain consistently and strongly with antibodies against ubiquitin and αB-crystallin, and positively, although not as intensely, with antibodies against α- and β-tubulin (1–3, 8, 14). Immunohistochemical staining with various anti-tau and anti-paired helical filament (PHF) antibodies have been reported to be weak to negative (1, 3, 8). Arai et al (14) and Abe et al (9) reported strong staining with a monoclonal antibody against microtubule-associated protein (MAP) 5 (MAP1B). Studies have been consistently negative with antibodies to actin, vimentin, desmin, cytokeratin, glial fibrillary acidic protein (GFAP), myelin basic protein, skeletal myosin, and neurofilament. The GCI-containing cells are immunoreactive with antibodies against oligodendrocyte-associated markers, including Leu-7, carbonic anhydrase isoenzyme II, and transferrin (1–3, 14).

To date there have been no reports of detailed biochemical analyses of these inclusions. Many of the electron microscopic studies of GCIs support the possibility that they are abnormal microtubular structures exhibiting a similar cross-sectional diameter, 25 nm, and morphologically appear as distinctively tubular cytoskeletal structures. Additionally, immunohistochemical analyses have shown fairly consistent immunoreactivity for both α- and β-tubulin. The variable reports of staining with MAP5 and tau antibodies may also indicate that microtubules in affected cells are abnormally associated with or stabilized by these microtubule-associated proteins (14). The presence of these MAPs in glial cells may be indicative of a pathological process such as increased expression or altered interaction with the microtubular cytoskeleton. Expression of MAPs within normal glial cells has been documented but the levels appear to be low (15, 16). The presence of αB-crystallin and ubiquitin implies a response to cellular stress, but what that might be is unknown (see below for a discussion of stress proteins in glia).

GCIs have been found only in cases of sporadic MSA. Familial cases of MSA do not appear to exhibit this pathology (2). No such inclusions have been reported in any of the normal controls or other neuropathologic disorders that have been examined as part of the various reported series. The GCIs of MSAs accumulate in the white and gray matter of the corticocerebellar, pyramidal, and extrapyramidal systems and the primary motor and higher motor areas of the cerebral cortex (17). The putamen, pallidum, lateral part of the caudate nucleus, internal capsule, basis pontis, middle cerebellar peduncle, white matter of cerebellar hemispheres, and the intermediate gray substance of the spinal cord have consistently been found to accumulate significant numbers of GCIs, while structures such as the substantia nigra, locus ceruleus, inferior olivary nucleus and dorsal motor nucleus of the vagus usually contain few or none (1). The numbers of inclusions seem to increase in parallel with the numbers of interfascicular oligodendroglia. In general, the distribution of glial pathology parallels the degeneration seen in the corresponding neuronal populations and white matter, but interestingly also involves regions that classically have been observed to be minimally or not involved. These areas include the external capsule, the lower cortical layers and subcortical white matter of the primary motor, premotor regions of the cerebral hemispheres, portions of the corona radiata, subthalamic area, and reticular formation of the lower brainstem. The pallor of myelin staining and the distribution of GCIs appears to be more widespread than the degeneration of nerve cells and axons. This raises a number of questions. Does the disease process in the MSAs first affect oligodendrocytes or neurons? Why do GCIs appear in the development of the disease—before or after neuronal degeneration? Are the MSAs diseases that specifically target oligodendroglial cells or are GCIs merely some reactive process secondary to neuronal damage?

The GCIs are more numerous and widespread than the neuronal inclusions in these diseases and can serve as specific markers. The intranuclear inclusions that have been reported in MSA have not been as widely accepted as a diagnostic feature. These have only been reported by the group of Papp and Lantos (11), without any other confirmation from the various other MSA researchers. Papp and Lantos have even stated that the nuclear inclusion is very similar to the nuclear inclusion observed in brains of normal controls.

Although there are many questions that remain unanswered concerning GCIs, it is clear that this distinctive

abnormal tau proteins. An astrocytic nucleus is clearly visible in the center of the tuft. PSP. Motor cortex. Monoclonal antibody PHF-1 staining, ×400. (f) Thorn-shaped astrocytes typically are located in subpial regions and show much more pronounced cytoplasmatic staining than tufted astrocytes. Motor cortex. PSP. Gallyas silver stain. ×300. (g) Coiled bodies wrap around cell nuclei and trail into processes. Argyrophilic threads are also present in the background. Red nucleus. PSP. Gallyas silver stain, ×340. (h) A large astrocytic "plaque" composed of a collection of argyrophilic, irregularly shaped particles. Much thinner argyrophilic threads are present in the background. CBGD. Gallyas silver stain, ×170. (i) Prominent argyrophilic threads are present within the gray matter of the motor cortex. Most of these are not associated with cell nuclei. An NFT is present in the lower left corner. CBGD. Gallyas silver stain, ×140. (j) A large number of Rosenthal fibers are present around a blood vessel. Cerebellar white matter. Alexander disease. H & E, ×100. (k) Small granular inclusions are present in the cytoplasm of an astrocyte in the subcortical white matter. Infantile Alexander disease. H & E stain, ×440. (l) Eosinophilic inclusions fill the cytoplasm of a cortical astrocyte in the occipital lobe. Alzheimer disease. H & E stain. ×310.
GLIAL FIBRILLARY TANGLES

Until recently tau protein and its related tangle pathology had been generally viewed as specific to neurons. Expression of tau protein in normal astrocytes and oligodendrocytes had only been rarely reported in rodent (15, 16) and human brain tissues (19). In the past several years, however, it has become quite evident that tau tangle pathology within glial cells, the so-called "glial fibrillary tangle", is present in many of the neurodegenerative disorders in which there is neuronal tangle pathology. In fact, judging from the present trend, one could predict that coexistent neuronal and glial tangle pathology will be the rule.

Progressive Supranuclear Palsy

Glial tangle pathology in PSP has recently received much attention (20–26). Distinctive and widespread tangle pathology appears to be a constant finding in PSP. The pathology can conveniently be grouped into three morphological types. Cortical and subcortical gray matter exhibit "tufted" astrocytes. Subpial, subependymal, and perivascular regions exhibit so-called "thorn-shaped astrocytes" (27). White and gray matter exhibit oligodendroglial cells bearing "coiled-bodies." Additionally, white matter tracts also exhibit "interfascicular" threads or processes, which are most likely glial in nature. The pathology is presently best visualized by the Gallyas silver stain and by immunohistochemical stains for various tau epitopes. The Bodian, modified Bielschowsky, and methenamine silver staining methods will react with these structures to varying degrees but are poor compared with the Gallyas stain. These structures cannot be seen with routine H & E, Kluver-Barrera, or Holzer stains. The presence of tau-positive astrocytes in PSP has recently gained enough acceptance among neuropathologists to be included as supportive diagnostic criteria under the preliminary NINDS neuropathologic criteria for PSP (28).

Tufted Astrocytes: The so-called "tufted astrocyte" is one of the most distinctive histopathologic features of PSP. These have been referred to as "tufts of abnormal fibers" (29), "starlike tufts of fibers" (20, 22) and "tufts of spiderlike radiating fibers" (30). With the Gallyas silver stain and immunohistochemical stains for various tau epitopes, these appear as discrete foci, or tufts, of radiating fibers (Fig. 1d, e). Within the center of these densestaining tufts, an astrocytic-appearing nucleus can be seen, especially in the immunohistochemical preparations (Fig. 1e). In silver-stained preparations the nucleus is often obscured by dense layers of fibers. The abnormal inclusions are detected mainly within the proximal and distal cytoplasmic processes; the perikaryal cytoplasm appears to be less involved. Tau antibodies against both normal and abnormal PHF epitopes consistently stain these tangles, while anti-ubiquitin antibodies are unreactive. The astrocytic nature of the affected cells has been confirmed by positive staining with antibodies against the astrocytic markers GFAP and CD44. Microglial and oligodendroglial markers are negative on these tufted cells (31).

Tufted astrocytes are relatively specific for PSP, with a few reports indicating possible overlap with CBD. In PSP, the tufted astrocytes are distributed in high density within the striatum, moderate density in the thalamus and in varying numbers in the cerebral cortices and brainstem (22, 23). The precentral gyrus is a fairly consistently affected area (25; Chin and Goldman, in preparation).

Thorn-shaped Astrocytes: The subpial zone of severely affected cortical regions, such as the precentral gyrus, midbrain, and brainstem frequently exhibit glial inclusions that are flame- or thorn-shaped (Fig. 1f). The argyrophilia and tau-immunoreactivity is localized mostly to the perikaryal cytoplasm with extension into the proximal portions of cell processes. Most of these processes are thick and short with only occasional longer ones, contrasting with the predominantly long and uniformly thick processes in tufted astrocytes. The immunohistochemical profile of thorn-shaped astrocytes is similar to that of the tufted astrocytes, with the exception of their reactivity with antibodies against ubiquitin, about which there is debate (21, 27). Thorn-shaped astrocytes are unreactive with antibodies against high molecular weight neurofilament protein and MAPs 1, 2, and 5 (27). An ultrastructural study of subpial white matter glial cells has demonstrated the coexistence of 15-nm straight tubules and intermediate filaments (27).

Thorn-shaped astrocytes are not specific for PSP. Ilesa et al (27) conducted a survey of thorn-shaped astrocytes in aged, neurodegenerative, and nondegenerative disease cases. They found that thorn-shaped astrocytes, in contrast to tufted astrocytes, have no apparent disease specificity. However, they found that thorn-shaped astrocytes occurred in cases in which there were also neuronal tau-related, cytoskeletal abnormalities. Thorn-shaped astrocytes also appeared to be intimately associated with postencephalitic parkinsonism of von Economo encephalitis and dementia pugilistica, and are also seen in a relatively smaller percentage of cases of senile dementia of the Alzheimer type, PSP, Pick disease with Pick bodies, and diffuse Lewy body disease as well as in 2 of their 12 aged controls. These 2 cases also had neurofibrillary tangles (NFTs) in the hippocampus. Interestingly, they did not observe the presence of thorn-shaped astrocytes in any
of their 8 cases of Alzheimer disease and concluded that
the severity of the cytoskeletal abnormality does not ap-
pear to govern whether or not thorn-shaped astrocytes are
present. Additionally, in most positive cases, there were
only a few thorn-shaped astrocytes found in localized
regions.

Although tufted astrocytes and thorn-shaped astrocytes
are morphologically recognizable entities, the pathol-
ological changes that produce these phenotypes may be sim-
ilar. Since these two changes occur in different astrocyte
populations (subpial vs intracortical), the different mor-
phologies may reflect differences between subtypes of
astrocytes.

Coiled Bodies: Coiled bodies were first described by
Braak and Braak (32) in a series of patients characterized
by adult onset dementia associated with argyrophilic
grains. Subsequently, this entity has been seen in a num-
er of neurodegenerative diseases including PSP, CBDG,
Pick disease, and subacute sclerosing panencephalitis
(33–35). The inclusion appears as a fine bundle of fila-
ments that is commonly coiled about a round nucleus and
extends into the proximal part of a cell process (Fig. 1g).
Occasionally, affected cells display two or three discrete
bundles encasing the cell nucleus, forming a ‘nuclear
cage.’ Ultrastructurally, coiled bodies appear to be com-
posed of bundles of filamentous or tubular structures
measuring 15 to 20 nm in diameter (34). In general,
coiled bodies are smaller than GCIs and are not as vol-
luminous as the inclusions seen in tufted astrocytes or
thorn-shaped astrocytes. Double immunostaining studies
have demonstrated that coiled bodies occur in oligoden-
drocytes and therefore have been referred to as ‘’oligo-
dendrogiial microtubular masses’’ (33). Cells with coiled
bodies can be costained with anti-tau and anti-C4d anti-
obodies, suggesting that some of the cells are complement-
activated oligodendrocytes (CAO) (see below).

Coiled bodies are present in many regions of the brain.
They are usually numerous within the midbrain, particu-
larly within the red nucleus and tectum, and basal gan-
glia. Variable numbers of coiled bodies can be seen in
the thalamus, subthalamic nucleus, striatum, amygdala,
and subcortical white matter (Chin and Goldman, in prep-
paration).

Interfascicular Threads: Myelinated white matter
tracts, particularly the “pencil” fibers of Wilson in the
striatum and internal capsule, commonly exhibit num-
"erous long, thin, wavy and corkscrew-shaped argyrophilic
threads in cases of PSP (30, Chin and Goldman, in prep-
paration). Most of these threads do not exhibit any obvious
relation to cell nuclei, but rare profiles lie adjacent to
oligodendroglial masses. Similar structures have also been
described in CBDG (30). These have been referred to as
“argyrophilic threadlike structures” (30), although we
prefer to use the shorter term “interfascicular threads.”
Relatively little is known about these structures other
than they are best seen with the Gallyas silver stain and
that they are immunoreactive with anti-tau antibodies but
not with anti-ubiquitin antibodies. It has been proposed
from a limited ultrastructural study that the interfascicular
threads originate from the inner and outer loops of oli-
godendroglia (30). These argyrophilic structures do not
appear to be specific for any one disease since we have
seen similar threads in cases of MSA, CBDG, and Alz-
heimer disease (Chin and Goldman, in preparation).

Corticobasal Ganglionic Degeneration

Corticobasal ganglionic degeneration is a disease entity
that can show both clinical and neuropathological overlap
with PSP (26, 36). Neurofibrillary tangles and tau-
immunoreactive pathology are present in neurons in the
basal ganglia and brainstem (37, 38). Recently, different
groups have reported differing ultrastructural observa-
tions of the neuronal tangles. Arima et al (39) reported
15-nm-wide straight tubules and long-periodicity con-
stricted tubules in neocortical neurons, Wakabayashi et al
(37) demonstrated 15-nm-wide straight tubules in nigral
neurons, and Kieszk–Reding et al (40) observed twisted
filaments with long-periodicity in fractions isolated from
frontal lobe tissue. Argyrophilic, tau-positive tangles
within glial cells are also found in CBDG (7, 26, 30, 36,
37, 41). There appear to be at least two distinct types of
glial inclusions in CBDG. One is morphologically similar
if not identical to the coiled bodies of PSP. These are
located predominantly in the frontal, pre- and post-central
cortices and in the white matter underlying the affected
cortex. Scattered inclusions have also been noted in the
deep white matter of the frontal and parietal lobes, corpus
callosum, internal capsule, basal ganglia, thalamus, mid-
brain,pons,medulla oblongata and cerebellar white mat-
ter. These inclusions are typically immunoreactive for tau
protein, but not for ubiquitin. Ultrastructurally the inclu-
sions appear to be located within oligodendrocytes and
are composed of straight tubules measuring 15 nm in
diameter (37).

Astrocytic “Plaques”: The other recently described

glial pathology is the so-called “astrocytic plaque” (26, 38).
Using double immunohistochemical staining and confocal
microscopy, Feny and Dickson (38) demonstrated that
distinctive amyloid-negative cortical plaques
were composed of collections of focal dilatations of distal
processes containing dense deposits of tau-immunoreac-
tive material. The cells that make up these placque-like
structures were immunoreactive for GFAP, CD44, and S-
100, confirming their identity as astrocytes. Additionally,
these cells were distinguished from other uninvolved ast-
rocytes by their characteristic strong staining with anti-
vimentin antibodies. These astrocytic “plaques” can also
be easily recognized with the Gallyas silver stain (24)
(Fig. 1h). Astrocytic plaques can easily be distin-

from tufted astrocytes when examined with thick vibra-
tome or frozen sections. However, ambiguity may arise
when examining silver or immunostained sections pre-
pared with thin paraffin sections, which will contain tan-
gentially cut portions of the astrocytic inclusions. How-
ever, from our experience, especially with the Gallyas
silver stain, this problem rarely arises (Chin and Gold-
man, unpublished). It has been proposed that this new
entity is relatively specific for CBGD (24, 26, 38). But
Nishimura et al (31) identified what appear to be astro-
cytic plaques in 3 of their 9 cases of PSF. Further studies
on a variety of neurodegenerative cases are needed to
resolve this issue.

White Matter Threads: Prominent numbers of argyro-
philic, tau-positive threadlike processes have been re-
ported in cases of CBGD (24, 26, 30, 38, 41). These
appear morphologically similar to the intercellular
threads described in cases of PSP, but differ in distribu-
tion and pattern, occurring as dense meshworks involving
frontal and parietal cortex and underlying white matter
(FIG. 11). It is suggested that these are oligodendroglial
in nature, but detailed studies need to be done.

Pick Disease

Until recently, Pick disease has not been considered a
disease characterized by prominent neuronal tangles. Hof
et al (42), in their morphological study, noted that NFTs
in cases of Pick disease occurred more frequently than
previously thought. Additionally, Pick body pathology
has been found to share abnormalities in tau cytoskeletal
proteins with those of NFTs in Alzheimer disease and
PSP (26, 43, 44). A number of reports have appeared in
the literature describing different aspects of glial pathol-
ogy in classical Pick disease (26, 44, 45). Yamazaki et al
(45) reported a patient with a 20-year history of Pick
disease with tau- and ubiquitin-positive intracytoplasmic
inclusions within astrocytes in the subcortical white
matter. At the light microscopic level these appeared as either
a single, compact, or argyrophilic globular mass or as
multiple small, dotlike inclusions. Ultrastructurally, the
inclusions were straight tubules of approximately 15 nm
diameter, very much resembling the straight tubules com-
prising the neuronal Pick bodies in this and other Pick
cases (43, 46). Unlike neuronal straight tubules, which
tend not to be arranged in any orderly fashion, the astro-
cytic straight tubules tended to aggregate into bundles.
Although they were found free in the astrocytic cyto-
plasm, these straight tubules did not appear to intermingle
with the existing glial intermediate filaments. The inclu-
sions in this particular case were located both within the
cell body and distal processes, especially perivascular
processes. The astrocytic pathology was most frequently
observed in the white matter underlying the middle and
temporal gyri, which were the most severely affected ce-
rebral regions. Feany et al (26) confirmed the presence
of similar pathology in their cases and noted that these
were relatively numerous in Pick disease but were not
seen in PSP or CBGD.

Yasuura et al (44) noted that tau-positive astrocytes,
similar to the tufted astrocytes seen in PSP, were present
in all 4 of their reported Pick disease cases. These were
argyrophilic with the Bielschowsky stain, and positively
immunoreactive with the monoclonal anti-tau antibodies,
Tau-2 and Alz-50. Double immunohistochemical staining
studies confirmed the astrocytic nature of the affected
cells, showing colocalization with GFAP but not for
HLA-DR or CNPase, markers for microglia and oligo-
dendroglia, respectively. Feany et al (26) noted that in
selected Pick cases, neocortical tau-immunoreactive ast-
rocytes appeared to equal or even outnumber Pick bod-
ies. The astrocytic inclusions in Pick disease were noted
to occupy more of the cell body than that of tufted ast-
rocytes and to ramify into the cell processes and extend
into the distal regions of the cell process. The tau-
immunoreactive inclusions also appeared to be localized
to one side of the cell, in contrast to the more symmetric
distribution of the inclusions in tufted astrocytes of PSP.
Feany et al also noted that the astrocytic inclusions in
Pick disease were not common in subcortical structures.
Unlike the cortical astrocytic inclusions in PSP, those
seen in Pick disease react with ubiquitin antibodies. How-
ever, inclusions seen in subcortical regions are largely
negative, showing only occasional weak immunoreactiv-
ity.

Both the cortical and white matter astrocytic inclusions
appear to be composed, at least in part, of abnormal tau
proteins. This has been shown by immunohistochemical
staining using a variety of anti-tau and anti-PHF antibo-
dies (43, 44). Delacourte et al (47) have recently shown
by Western blotting analyses that the tau protein profile
of brain tissue from Pick disease is largely composed of
isoforms running as a doublet at 55 and 64 kDa in one-
dimensional polyacrylamide electrophoretic gels. This
pattern is distinctly different from that seen with the
straight filaments of PSP. However, from this type of
analysis, one cannot determine whether the protein pro-
files are similar for both the neuronal Pick bodies and the
astrocytic inclusions.

Another glial pathology seen in Pick disease is the presen-
tce of CAOs, which are detected by immunostaining
with antibodies against C3d, C4d and amyloid P com-
ponent (33, 44). The CAOs were seen in increased numbers
in severely affected cortical areas in advanced cases of
Pick disease. Fibers similarly positive for C4d and am-
loid P component were also found in high density in the
stratum lacunosum of the hippocampus in these ad-
vanced cases. Within the stratum granulosum of the den-
tate fascia, clusters of immunoreactive granules were
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seen with these antibodies and were found to have apparent continuity with CNPase-immunoreactive oligodendroglial fibers. These C4d and amyloid P component immunoreactive structures may represent CAOs in the hippocampal formation. Yasuhara et al. (44) noted that C4d-immunoreactive granules and hypertrophied CAOs in the dentate fascia appeared to be a relatively specific finding in that they did not find these changes in any cases other than those with Pick disease, although their sample size was relatively small.

Alzheimer Disease

Tau immunoreactivity in glial cells of Alzheimer disease cases has been reported. Both astrocytes (48–51) and oligodendrocytes (52) are affected. The oligodendrocyte pathology appears to be identical to the “coiled body” (see above). Interestingly, both the astrocystic and oligodendrocytic inclusions in Alzheimer disease have been found to contain not only straight filaments, common to the above described “glial fibrillary tangle” types, but also PHFs. The significance of this observation is unclear at this time, but it is tempting to speculate that the pathogenesis of the glial cell pathology is very similar to that of neurofibrillary tangles.

Other Neurodegenerative Diseases

Coiled bodies have been described in cases of dementia associated with argyrophilic grains (32, 33, 53) and in subacute sclerosing panencephalitis (34, 35). Tufted and thorn-shaped astrocytes have been demonstrated in cases of postencephalitic parkinsonism of von Economo encephalitis (54).

HEAT SHOCK PROTEIN INCLUSIONS

Several inclusions are composed in part of heat shock proteins. These include Rosenthal fibers (RF), eosinophilic granular bodies, cysteamine-induced inclusions, and eosinophilic inclusions of gray matter astrocytes seen in patients with Aicardi syndrome. We are grouping these various inclusions under the general category of heat shock protein inclusions, since they share this feature, but bear in mind that each contains other proteins as well.

Rosenthal Fibers

RFs are eosinophilic, cytoplasmic inclusions of astrocytes. Initially described in the giotic walls of syrinx cavities, RFs also accumulate in pilocytic astrocytomas and occasionally in astrocytic scars in multiple sclerosis plaques and chronic infarcts (55, 56). The most dramatic example of RF formation occurs in Alexander disease, in which massive numbers accumulate throughout the CNS in subpial astrocytes, white matter, brain stem, and cerebellum (55, 57, 58) (Fig. 1J). They do not appear to be any morphological or compositional differences between RFs in Alexander disease and those in other disorders. RFs vary in size from round, focal deposits of a few microns to elongated, cigar-shaped fibers one hundred microns or more in length for those that reside in astrocyte processes. In Alexander disease, they first appear in cell bodies as small, focal inclusions (Fig. 1K), and with time, accumulate at the end feet of astrocytes, resulting in their preferential localization around blood vessels and at pial surfaces (Fig. 1J). White matter and subpial astrocytes accumulate RFs far more strongly than cortical or deep gray matter astrocytes in Alexander disease.

The ultrastructural appearance of a RF is that of a dense, osmiophilic mass lying on a meshwork of intermediate filaments (55). The dense part of the inclusion is composed of two small molecular weight heat shock proteins, αB-crystallin and hsp27 (59). Some of the αB-crystallin is conjugated to ubiquitin (60). The filaments represent both GFAP and vimentin (61).

Why small MW heat shock proteins accumulate in astrocytes in these various conditions is not known. A variety of “stresses,” including elevated temperature, hypoxia, hypoglycemia, heavy metal exposure, hypertonicity, and cytokines upregulate αB-crystallin gene transcription and translation in astrocytes (62), but what specific insults cause RF formation in human pathology is not yet known.

Two biochemical properties of αB-crystallin and hsp27 are likely to be important in producing RFs. First, both proteins are self-aggregating. For example, αB-crystallin in the lens and in nonlenticular tissues normally resides as multimeric aggregates of 35 to 40 molecules (63–65). Second, in addition to their proclivity to aggregate, these heat shock proteins display an affinity for intermediate filaments (see Bennardini et al. [66]). Indeed, a small fraction of αB-crystallin in astrocytes is normally bound to intermediate filaments (Wisniewski and Goldman, unpublished observations). Thus, RFs reflect the natural affinities of small heat shock proteins for each other and for intermediate filaments. Why aggregates accumulate is not known. In Alexander disease, examination of infantile patients has shown small RFs primarily in astrocyte cell bodies (58), whereas the RFs in older Alexander patients are typically large and found mainly in astrocyte processes and in astrocyte endfeet. This difference suggests a progression, or evolution, in which RFs begin as small aggregates in cell bodies and then grow or coalesce into larger aggregates as they are transported along processes to endfeet. The reasons for aggregation are not yet known. Levels of αB-crystallin and hsp27 mRNA are elevated in Alexander disease (67), consistent with transcriptional activation of stress protein genes. However, as the inclusions enlarge, they may become more and more resistant to the normal cellular turnover mechanisms. The ubiquitination of αB-crystallin
would then represent an attempt to metabolize the inclusions. Thus, post-translational stability may play a role in the generation of RFs.

**Eosinophilic Granular Bodies**

The eosinophilic granular bodies and hyaline droplets of astrocytomas also contain αB-crystallin and hsp27, as judged by immunocytochemical studies (68, 69). They appear ultrastructurally as round, dense, cytoplasmic inclusions. They do not display the bundles of intermediate filaments that exist in RFs.

**Eosinophilic Inclusions in Cortical Astrocytes**

Several reports have described small, eosinophilic inclusions in astrocytes in patients with Aicardi’s syndrome and other types of mental retardation (70–72). Although at the light microscopic level they bear a resemblance to RFs, there are several important differences. These inclusions do not contain intermediate filament bundles. They display a granular appearance (73), but not one as dense as that of RFs. Furthermore, their distribution in the CNS is different from that of RFs, being found in neocortical astrocytes and not in subpial, white matter, or subcortical gray matter astrocytes. Indeed, the distribution appears to be the reverse of RFs. Although not originally described to contain heat shock proteins, recent immunocytochemical studies show both αB-crystallin and hsp27 in these inclusions (ZuRhein et al, in preparation). In addition, antibody studies show the presence of S-100β and GFAP in some.

Only a few such patients have been described, but it is likely that the inclusions are present in a wider variety of ages and clinical syndromes. For example, Wiegol and Wisniewski (74) and Spacek and Nozicka (75) describe them in the neocortex of patients with Alzheimer disease and fibrohyaline vasculopathy and the latter authors even note an early report by Alzheimer himself that appears to describe similar inclusions. We have also seen the rare Alzheimer patient with many such inclusions in the neocortex (Chin and Goldman, unpublished observations) (Fig. II).

**Autofluorescent Astrocyte Inclusions**

Astrocytes that populate the periventricular regions of the CNS are prone to accumulate autofluorescent, Gomori-positive inclusions during aging (reviewed in 76). Such inclusions are increased after irradiation and prolonged estrogen treatment. The spectral characteristics and the presence of endogenous peroxidase activity suggest the presence of heme iron and porphyrin. The inclusions also contain the stress proteins hsp27, glucose-regulated protein, and ubiquitin (77). Inclusions with similar spectral, enzymatic, and immunocytochemical characteristics can be induced in rat astrocytes in vivo and in culture by the application of cysteamine, a sulfhydryl reducing agent, or hydrogen peroxide (78). Schipper has argued that a chronic oxidative stress produces these inclusions and that the mechanism involves the production of free radicals from cellular metabolites such as catecholamines and indolamines and their catalytic products. Such products might be expected to accumulate during the course of aging.

**PERSEPCTIVES**

The recent realization that glial tangle pathology is a very common feature of CNS degenerative diseases classically associated with prominent neuronal cytoskeletal abnormalities underscores the widespread involvement of both neurons and glia. Whatever the etiological causes of the various diseases may turn out to be, it is clear that both neurons and glial cells share common cytoskeletal pathologies. Could this be evidence that tau pathology is indeed some common reactive process that occurs somewhere downstream of an initiating insult, or as some sort of common adaptive response to the continued presence of some unidentified toxin (cytokine)? Support for such a hypothesis comes from the observation that the viral-associated neurodegenerative diseases, postencephalitic parkinsonism of von Economo encephalitis and subacute sclerosing panencephalitis, also exhibit tangle pathology in both neuronal and glial populations. Indeed, if neurons and glial cells share similar pathogenetic pathways toward tangle pathology, in vitro systems using glial cells will be a reasonable way to study dynamic aspects of these disorders.

The presence of stress proteins and tau in a large number of diverse glial inclusions is noteworthy. The “stresses” that result in such upregulation are not clear, however. Some could be a response to the accumulation of abnormal proteins, such as modified forms of tau. Others could be a direct result of some form of metabolic stress, such as seen in cysteamine-mediated accumulation of stress proteins. It is important to realize that a number of different “stresses” can regulate classes of proteins, including oxidative and thermal stress, heavy metals, low glucose, hyper- and hypo-osmolality, and a number of cytokines, including TNFα. The precise constellation of stress proteins that accumulates may give clues as to the initial insult, but there is extensive overlap in stress responses.

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