Rabies-Like Neuronal Inclusions Associated with a Neoplastic Reticulosis in a Dog

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Abstract. Neoplastic reticulosis of the central nervous system was associated with intracytoplasmic inclusion bodies in Purkinje cells and neurons of several nuclei. These eosinophilic inclusion bodies were from round to crescentic and had a different location, structure, and cytochemistry than Negri bodies and inclusions of other canine neurologic diseases. The inclusions represent an aberration of rough endoplasmic reticulum and resemble the cytoplasmic laminar bodies of the lateral geniculate neurons of the cat.

Few neoplastic reticuloses of the central nervous system of dogs have been described [3]. None has been reported in which there was an apparent association with cytoplasmic inclusion bodies in neurons. The inclusion bodies resembled Negri bodies, and since fresh tissue for animal inoculation or fluorescent antibody techniques [5] was not available, histochemistry and electron microscopy were used in the differential diagnosis.

Materials and Methods

A 6-year-old female Cocker Spaniel was seen by a veterinarian because of restlessness and continuous walking. She was examined again 3 days later because of marked depression. A blood sample was taken for a complete blood count and certain serum chemical determinations. The dog died 4 days after the initial visit.

Formalin-fixed tissues submitted to our laboratory included pancreas, adrenal, esophagus, stomach, kidney, liver, lung, urinary bladder, small and large intestine, spleen, heart, uterus, and brain.

Staining was done on 6-μm sections of paraffin-embedded tissue except for Sudan black B for which frozen sections were used. The staining methods used to study the inclusion bodies are summarized in table I. Additional stains for study of the infiltrative cells were Gomori’s methenamine silver and Masson’s trichrome [8].
Table I. Histologic technics

<table>
<thead>
<tr>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin and eosin [8]</td>
<td>eosinophilic</td>
</tr>
<tr>
<td>May-Gr&quot;unwald Giemsa [8]</td>
<td>pink</td>
</tr>
<tr>
<td>Periodic acid-Schiff [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Sudan black B [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Birefringence [15]</td>
<td>negative</td>
</tr>
<tr>
<td>Luxol-fast blue [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Feulgen [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Shorr S3 [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Toluidine blue pH 3.0 [15]</td>
<td>not metachromatic</td>
</tr>
<tr>
<td>Ziehl-Neelsen acid fast [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Congo red with polarization [15]</td>
<td>negative</td>
</tr>
<tr>
<td>Mallory's phosphotungstic acid-hematoxylin [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Mucicarmine [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Massignani-Malferrari [8]</td>
<td>negative</td>
</tr>
<tr>
<td>Lentz [6]</td>
<td>negative</td>
</tr>
</tbody>
</table>

Selected areas of formalin-fixed cerebellum and basal ganglia were prepared for electron microscopy. After washing overnight in 0.3 M sucrose solution in 0.05 M S-collidine buffer, pH 7.4, the tissues were fixed in S-collidine-buffered 1% osmium tetroxide for 90 min, dehydrated in graded ethanols, embedded in epon 812, and polymerized at 60 °C. Sections 1 μm thick were cut and stained with toluidine blue for light microscopy. Suitable blocks were sectioned at approximately 600 Å on an LKB ultramicrotome with glass knives, stained with a saturated solution of uranyl acetate in 50% ethanol and 0.2% lead citrate, and examined in a Hitachi II A electron microscope.

Results

There were marked perivascular cellular infiltrations of the meninges, choroid plexus, cerebrum (fig.1), including the hippocampus, and the anterior parts of the brain stem. The posterior brain stem was less severely affected. Generally, the white matter was infiltrated to a greater degree than the grey and at times was vacuolated. The infiltration of the cerebellar meninges was mild and patchy. There was little infiltration of the cerebellum.

The cells making up the perivascular cuffs (fig. 2) and meningeal and parenchymal infiltrations included relatively large mononuclear cells that were considered neoplastic reticulum cells, small to large lymphocytes and
neutrophils. The population of these cells varied in different sites. Neoplastic reticulum cells were prevalent in perivascular cuffs, less often in white matter, and rare in grey areas. They were recognized by their relatively large size, clumped chromatin pattern, prominent single or multiple nucleoli, and fine
reticular fibers at the cell surface as demonstrated with Gomori’s methenamine silver stain. These cells at times appeared larger than normal reticulum cells, somewhat bizarre, and displayed mitotic activity. Mature lymphocytes usually were present in cuffs only. The medium to large lymphoid cells were in cuffs and parenchymal areas. Necrotic cellular debris and occasionally neutrophils were present in the infiltrated areas.

Eosinophilic intracytoplasmic inclusion bodies frequently were present in Purkinje cells of the cerebellum and in neurons of certain nuclei. The nuclei with neuronal inclusions included the lateral geniculate body, pulvinar, the superior colliculus and the basal ganglia. Inclusion bodies were not detected in the cerebral cortical areas nor the hippocampus.

Most of the inclusion bodies were near the plasma membrane and were ovoid to crescentic (fig. 3); some were round. They were usually about 3–9 μm in diameter and occasionally 14 μm in length. Some cells had one to three inclusions, most of which were segmented; a few inclusions had partial halos. The inclusions lacked basophilic inner granules, which occur in Negri bodies, and had a laminated appearance.

Staining results are summarized in table I. Most staining was negative but with hematoxylin and eosin the bodies were eosinophilic, and with May-Grünwald-Giemsa they were pink.
Fig. 4. Part of a Purkinje cell. Two prominent inclusions and numerous smaller aggregates of parallel structures (example at arrow). N = nucleus; RER = rough endoplasmic reticulum.

Fig. 5. Small lamellar inclusions in close association with rough endoplasmic reticulum (RER).
Fig. 6. Presumptive early stage in the formation of lamellar inclusion. Fusion (arrow) of two segments of rough endoplasmic reticulum (1 and 2) along apposing ribosomal surfaces.

Fig. 7. Part of a Purkinje cell. Several small lamellar inclusions illustrating granular matrix enclosed by unit membranes (L); a unit membrane uniting endoplasmic reticulum to lamellar element (arrow at top); segments of outer nuclear membrane resembling lamellar inclusion elements (arrow, lower left). N = nucleus; M = mitochondria.
Ultrastructurally, Purkinje cells contained one or two ovoid or crescent-shaped prominent cytoplasmic inclusions and many smaller inclusions throughout the cytoplasm (fig. 4). The inclusions were more electron dense than adjacent cytoplasmic areas and consisted of aggregates of intermittently interconnected parallel and shorter branched structures bounded by unit membranes and enclosing a granular matrix of uneven electron density (fig. 5). These structures frequently appeared to be continuous with segments of rough endoplasmic reticulum and were within cisternal elements (fig. 5). In some places it seemed that individual elements of the lamellar inclusions were formed by fusion of two segments of rough endoplasmic reticulum along apposing ribosomal surfaces (fig. 6). Some small inclusions were adjacent to and appeared to include segments of the outer portion of the nuclear membrane (fig. 7).

Discussion

The neoplastic reticulum cells described here are comparable to the cells in the cases presented by Fankhauser et al. [3] as neoplastic reticuloses. The small lymphocytes that were generally present only in cuffs may represent the cellular response engendered by the neoplastic process. The medium to large lymphoid cells in cuffs and parenchymal areas may also represent such a response or may themselves be a component of the neoplastic process.

Nonsuppurative encephalitis of rabies typically is a perivascular cuffing of lymphocytes and diffuse and focal gliosis of the grey matter [5]. The cellular reaction is generally less severe than seen in this case, and both the infiltrative cells and distribution of the lesions are different.

Old dog encephalitis [1,7] and canine distemper [4,13] also have different populations of reactive cells, parenchymal changes and distribution of lesions than those described here. The lesions of the central nervous system in infectious canine hepatitis are essentially secondary to vascular injury [4]. Herpesvirus infection in dogs is characterized by a nonsuppurative encephalomyelitis with focal malacia [13]. The inclusion bodies seen in the brain in canine distemper, old dog encephalitis, infectious canine hepatitis and herpesvirus infection of dogs usually are intranuclear.

The inclusion bodies described here differ from Negri bodies in regard to neurons affected and light- and electron-microscopic features. They were found primarily in the Purkinje cells of the cerebellum and certain nuclei...
of the brain stem and were not present in the hippocampus. The inclusion bodies of rabies in dogs are most readily found in the neurons of the hippocampus [13]. Negri bodies are usually round, often have halos, usually are 2–8 μm in diameter, and may contain inner basophilic bodies when stained with Mann's stain and viewed with the light microscope [13].

Electron microscopy did not reveal virions or subvirions. The cytoplasmic inclusions were unequivocally distinguishable from Negri bodies, which are characterized by a homogeneous granular matrix displacing cytoplasmic components of nerve cells. Clumps of rod and bullet-shaped rabies virions are found within swollen vesicles of the endoplasmic reticulum within or contiguous to the granular matrix [10].

There are certain similarities between the bodies described here and those reported as cytoplasmic laminar bodies in the lateral geniculate neurons of the cat [2, 12, 14], and Loewenthal's bodies in the thalamus of cats [9]. The ultrastructure of feline laminar bodies was initially described by Morales et al. [11]. Information on the ultrastructure and histochemistry of these structures was extended by Doolin et al. [2], who concluded that they are derived from endoplasmic reticulum.

The cytoplasmic inclusions in neurons reported here appeared to represent an aberrant formation of the rough endoplasmic reticulum. We suggest that cisternal elements of the reticulum fuse along their ribosomal surfaces, resulting in aggregates of modified lamellar structures. The fusion of two segments of reticulum yields a product bounded on each side by a unit membrane enclosing a granular matrix (fig. 6).

Acknowledgements

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References

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