Spontaneous Listeric Encephalitis in Sheep

Electron Microscopic Studies

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Abstract. The brainstems of four sheep with spontaneous listeric encephalitis had scattered small foci of inflammatory cells (neutrophils or macrophages, or both) with scattered fragments of degenerating nerve fibers and glial cells. In extensive areas of malacia in the pons and medulla oblongata, there was loss of parenchyma with massive accumulation of macrophages, a few neutrophils, lymphocytes and plasma cells. In both types of lesions, phagocytes contained debris of myelin and axons, lipid vacuoles and occasionally bacteria. Neutrophils contained bacteria in phagocytic and digestive vacuoles. No bacteria were detected in macrophages but were detected in neurons and in one axon in tissue previously used for paraffin sections.

A cardinal feature of listeric encephalitis in sheep is many nodules of macrophages or neutrophils or both in the parenchyma of the brain stem, especially the pons and medulla oblongata. The proportion of macrophages to neutrophils in these inflammatory foci varies greatly in the same brain. Also, the predominant type of inflammatory cell (macrophage or neutrophil) varies between different brains [4]. In light microscopic studies, bacteria occurred more frequently in neutrophils than macrophages [4]. No reason was found for this apparent greater phagocytosis by neutrophils. Although the importance of cell mediated immunity in listeric infections is well established [16], there are no reports of the ultrastructural features of the inflammatory response and phagocytosis of bacteria in listeric encephalitis.

Experimental studies indicate that some proteins and viruses (herpes simplex virus, infectious bovine rhinotracheitis virus, pseudorabies virus, canine herpes virus) travel to the brain via axons of peripheral nerves [3, 5, 7–11, 17, 18]. Viruses also may spread within the brain via neuronal processes [7]. A recent study of listeric encephalitis in sheep suggested that bacteria migrated along peripheral nerves to the brain and that migration in the brain occurred, at least partly, along fiber tracts [4]. A few neurons and neuronal processes contained organisms which suggested that neuronal cytoplasm is a vehicle for movement of the infectious agent [4].
This study was undertaken to study the ultrastructural features of the inflammatory response and intraneuronal bacteria in spontaneous listeric encephalitis in sheep.

**Materials and Methods**

Seventeen sheep with listeric encephalitis were necropsied [4]. Tissues from four were used for electron microscopic studies. Immediately after two sheep were killed pieces of medulla oblongata were fixed in 2 percent glutaraldehyde in Millonig’s buffer [19]. The entire brain from each of two other sheep was fixed in 10 percent neutral buffered formalin. Pieces of brain, fixed by both methods, were fixed in osmium tetroxide and embedded in epon. In a previous examination of Giemsa-stained paraffin sections, bacteria were detected in neurons and neuronal processes [4]. Pieces of some of these sections were fixed in osmium tetroxide and embedded in epon. Sections, cut at 1 micrometer, were stained with toluidine blue and examined with a light microscope; thin sections were stained with lead citrate and uranyl acetate and examined with an electron microscope.

**Results**

In extensive areas of malacia in the medulla oblongata and pons, there were massive accumulations of macrophages with a few neutrophils, lymphocytes and plasma cells. In addition to inflammatory cells, there was extensive replacement of normal brain parenchyma by fluid containing fragments of degenerating nerve fibers, fragments of unidentified cells and a few erythrocytes (fig. 1). Macrophages had many cytoplasmic projections and contained phagocytosed myelin and axons, tubular and lamellar dense bodies, clear vacuoles and lipid vacuoles. Plasma cells and lymphocytes occurred more frequently adjacent to blood vessels. Endothelial cells of some blood vessels contained many pinocytotic vesicles and surface invaginations. A few swollen nerve fibers had dense accumulations of degenerating mitochondria, homogeneous dense bodies and lamellar profiles. A few swollen myelinated fibers contained neutrophils.

All of the small foci of inflammatory cells that were examined had mostly neutrophils. Occasionally the phagocytes were scattered among intact neural cells, but usually they were adjacent to and had phagocytosed fragments of degenerated brain parenchyma. In nodules of inflammatory cells, bacteria were detected in phagocytic vacuoles of scattered neutrophils (fig. 2). Most of the phagocytosed bacteria had distinct cell walls and internal membrane systems. There was abundant peribacterial space. Neutrophil granules frequently were in contact with membranes of phagocytic vacuoles. This suggested imminent fusion (fig. 2). Some damaged bacteria were surrounded by moderately dense granular or fibrillar material in digestive vacuoles (fig. 3). Damaged bacteria had thin or misshapen cell walls and occasionally part of the cell wall was absent. Membranes of a few digestive vacuoles were fenestrated, and vacuolar membranes were not detected around some focal cytoplasmic accumulations of granular material associated with bacteria. No bacteria were detected in macrophages.

Although there was marked disruption of many ultrastructural features of brain parenchyma previously embedded in paraffin, the organisms were fairly distinct. They occurred alone and in pairs in neuronal perikarya and axons (fig. 4). No
Fig. 1: Macrophages in area of malacia in the medulla oblongata. Many phagocytic vacuoles contain fragments of myelin and axons. Intercellular debris.

bacteria were detected in the intercellular spaces. No ultrastructural details of adjacent cytoplasm could be seen because tissue had been distorted by previous paraffin embedding.

Discussion

The results of this study confirm that there are bacteria in neurons and their processes in ovine listeric encephalitis [4]. No previous reports of intraneuronal or intraaxonal bacteria in listeric encephalitis were found. In leprosy, intraaxonal bacteria may migrate within the axon and may contribute to nerve fiber destruction. Mycobacterium leprae occurred singly and in clusters in axons of peripheral nerves [20] and were free or within vacuoles in the axoplasm. Many of the axons with M. leprae were morphologically normal which suggested that bacilli in axons
were not merely an end-stage phenomenon [20]. Unfortunately, ultrastructural details of cytoplasm adjacent to *Listeria monocytogenes* were unidentifiable in the present studies because of previous paraffin embedding. By light microscopy, some of the affected neurons and their processes were intact [4] which suggests that *L. monocytogenes* may invade normal neurons.

The inflammatory response consisted of various inflammatory cells including macrophages, neutrophils, plasma cells and lymphocytes. Phagocytosed bacteria in different stages of digestion in neutrophils suggests that these cells were capable of destroying the organisms. Although studies of the sequential stages in phagocytosis and digestion of *L. monocytogenes* were not made, several of the stages seen ultrastructurally were similar to stages in the digestion of listeria and other bacteria by neutrophils [2, 6].

Studies of phagocytosis and digestion of *L. monocytogenes* by mouse peritoneal macrophages *in vitro* indicate that there is an initial esotropic uptake of bacteria with formation of phagocytic vacuoles and then accumulation of the contents of cytoplasmic vesicles (lysosomes) within the phagosomes [14, 15]. The general response was similar in normal and immune macrophages but seemed to proceed at
a faster rate in immune macrophages [15]. In macrophages of infected mouse spleen, *L. monocytogenes* accumulated in phagocytic vacuoles that frequently had electron-dense material derived from lysosomes [1]. Focal damage to the host cell occurred adjacent to damaged vacuolar membranes [1]. Breakdown of *L. monocytogenes* in rabbit alveolar macrophages began within 3 hours [12]. Fine granular or fibrillar material was seen around the intracellular organisms both inside and outside of the phagosome [12]. In addition to reports of intracellular digestion of listeria, some studies indicate that multiplication may occur in macrophages *in vitro* [1, 13, 16]. No bacteria were detected in macrophages in brains of sheep in this ultrastructural study, and in a previous light microscopic study [4] there were only a few scattered macrophages which contained organisms. These findings may indicate that macrophages failed to phagocytose and destroy *L. monocytogenes*, or that by the time sheep were killed most of the phagocytosed organisms had been reduced by intracellular digestion to debris no longer identifiable as bacteria. The latter suggestion seems unlikely because of the large number of intact bacteria seen with the light microscope in brains of some sheep [4]. Further investigations, including studies of the sequential development of lesions and the immune status of

*Fig. 4:* Several bacteria in cytoplasm of neuron or neuronal process. Two bacteria (arrow). Thin section of Giemsa-stained paraffin section (inset).
experimentally infected sheep will be required to determine the role of macrophages in ovine listeric encephalitis.

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References


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