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ULTRASTRUCTURAL CHANGES IN CNS AXONS IN EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS

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Amyotrophic leukospongiosis (AL) is a distinctive progressive spinal amyotrophy that is inevitably fatal, which belongs to the group of spongiform encephalopathies, caused by a non-classical virus [2]. In guinea pigs in which the infection is produced, disappearance of neurons (mainly spinal motoneurons), proliferation of astrocytes, and death of axons without any evidence of their demyelination are observed. Disturbances of the blood-brain barrier and penetration of immunocompetent blood cells into the CNS have not been observed [2, 3].

In the investigation described below, conducted on guinea pigs with experimental AL, the white matter of the spinal cord was studied in order to establish the specificity of degeneration of the central axons; other features studied included the response of the glial component and functioning of oligodendrocytes, astrocytes, and microglia as phagocytes, i.e., their role in the distinctive form of immunologic surveillance in a barrier organ such as the CNS.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs weighing 250-300 g, into which the liquid phase of a 10% suspension from the brain of patient D., dying from AL, was injected. The method of preparing the homogenates and of infecting the animals was described previously [4]. Three groups of animals were used in the experiments. In group 1 the nine guinea pigs were infected by the retro-orbital route in order to preserve the integrity of the blood-brain barrier. With this method of infection, clinical manifestations of the disease such as loss of hair, muscle atrophy, the development of pareses and paralyses of the limbs and trunk, oc-

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curred in the 5th-7th week (35th-40th days). From this group three animals were taken for investigation on the 14th, 21st, and 25th days after infection respectively. Group 2 consisted of nine guinea pigs which were inoculated intracerebrally with the liquid phase of a 10% brain suspension from the same patient. Clinical manifestations of the disease in this case developed in the 12th-18th week (90th-128th days) after infection. The 3rd (control) group consisted of six animals, of which three were inoculated by the retrobulbar route and three were inoculated intracerebrally with the supernatant of a 10% brain suspension from a clinically healthy person dying in an automobile accident. The animals were perfused with 4% glutaraldehyde solution in phosphate buffer (pH 7.2), the spinal cord was removed, and pieces of white matter from the anterior, posterior, and lateral columns were excised from the cervical, thoracic, and lumbar regions of the cord. These were then treated and embedded in Araldite by the usual method. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-100 CX 11 electron microscope. To avoid re-examination of the cells, only one section was analyzed in a series of sections obtained from the same block. About 3600 axons were studied and counting was done under a magnification of 6000, whereas ultrastructural changes were analyzed under a magnification of 80,000-90,000.

EXPERIMENTAL RESULTS

On the 14th day after infection changes were discovered in two of the three animals of group 1 in 6-10% of axons on electron-microscopic investigation. Most of them became paler and fewer neurofilaments were present in the axoplasm; single vacuoles and a fine precipitate appeared. Some increase in size of the periaxonal space was noted. Other axons appeared darker as a result of an increase in the number of neurofilaments, hypertrophy of the mitochondria, and increased electron density of the axoplasm. At this stage of experimental AL no phagocytosis of myelinated axons by any types of glial cells could be observed.

On the 21st day ultrastructural changes were found in 20-30% of axons in all three guinea pigs studied. In most of them (up to 65%) clarification of the axoplasm and destruction of neurofilaments and other subcellular structures were observed (Fig. 1a). The axoplasm became structureless in appearance, and sometimes only single collapsed mitochondria, vacuoles, and fragments of membranes were located in it. Widening of the intralamellar spaces and the appearance of intramyelin vacuoles with pale contents were clearly revealed. Sometimes the intermediate lines became interrupted and in some regions of the myelin sheath they disappeared; destruction of the principal dense lines was less frequently observed. In 30-42% of processes showing changes, shrinking of the axons, increased osmiophilia of the axoplasm, and the formation of large periaxonal cavities, often containing detached or separated fragments of myelin sheath, could be seen (Fig. 1b). The neurofilaments in the shrunken axons were destroyed or were unevenly grouped in structures occupying a central or peripheral position. Occasionally axons whose lumen was filled with concentrations of mitochondria, neurofilaments, and membranous structures, and with osmiophilic, often laminated inclusions, were found. The cytoplasm of some oligodendrocytes appeared pale and vacuolated, with destroyed mitochondria. A phenomenon of insinuation of astrocytic processes, filled with gliofibrils, between the separated lamellae of myelin was observed. Accumulation of lysosomes and the formation of large phagolysosomes in the cytoplasm of the astrocytes were not observed.

On the 35th day degenerative changes in the white matter of all animals of this group were even more marked. Many dying axons with relatively intact or a completely unraveled myelin sheath were observed. Myelin ovoids and spheres were frequently seen. Sometimes astrocytes phagocytosed the cell debris and their cytoplasm contained large myelin fragments, which preserved their lamellar structure, together with, less frequently, phagolysosomes. Lipid-containing inclusions, osmiophilic amorphous granules, and myelin structures were found in the cytoplasm of the oligodendrocytes. Some oligodendrocytes formed elongated pseudopodia, which phagocytosed processes of dying axons (Fig. 1c). Microgliocytes occasionally had a dilated perinuclear space and swollen cisterns of the rough endoplasmic reticulum. Neither large lipid inclusions nor phagolysosomes could be observed in their cytoplasm.

During investigation of six guinea pigs at the height of manifestation of the clinical features of the disease (70th-128th day after intracerebral infection) changes in the ultrastructure of the white matter mainly corresponded to those described after retrobulbar infection. Participation of blood mononuclears in the development of the degenerative changes was not observed. However, the glial reaction in the CNS was more marked. Besides large hypertrophied astrocytes, paired astrocytes, located side by side, appeared. The cytoplasm of individual astrocytes was filled with spirally coiled lamellae of myelin. In some areas of white

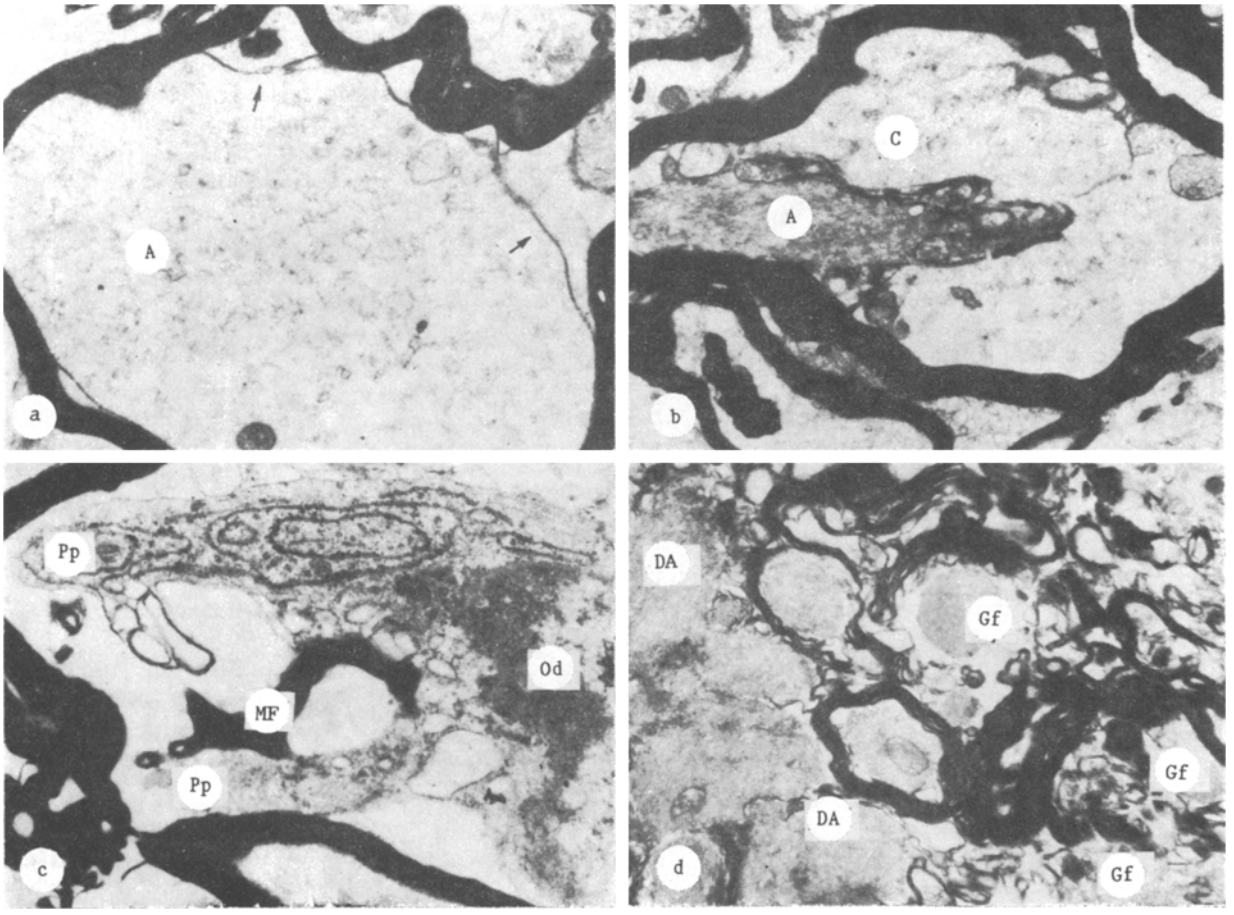

Fig. 1. Ultrastructural changes in white matter of spinal cord of guinea pigs with experimental amyotrophic leukospongiosis: a) pale axon (A) with completely destroyed neurofilaments and microtubules, mitochondria and axolemma (arrows) are preserved; b) dark, shrunken axon (A) with large periaxonal cavity (C): c) formation of pseudopodia (Pp) by oligodendrocyte (Od) and phagocytosis of myelin fragments (MF); d) area of total death of processes (bottom right corner), adjacent to which run demyelinated axons (DA); numerous astrocytic processes containing gliofibrils (Gf) can be seen here also. Magnification: a and b) 9000, c and d) 12,000.

matter with extensive foci of death of the processes demyelinated axons surrounded by astrocytic processes were observed (Fig. 1d). The axoplasm of these processes was filled with many neurofilaments. Proliferation of oligodendrocytes and phagocytosis of myelin sheaths by them were clearly manifested. The number of axons with two myelin sheaths was increased.

In the control group of animals no ultrastructural changes could be found in their CNS. These investigations showed that in the majority of infected animals, with the development of the pathological process the number of affected axons increased; degenerative changes took place mainly in accordance with the pale type. Marked phagocytic activity and absence of a myelinotoxic effect of the oligodendroglia were noted. The astrocytes also were able to ingest destroyed processes, although it is not clear whether phagocytosis in these cells was complete. It is possible that the inclusions observed in the cytoplasm of the astrocytes was a manifestation of dystrophy — they were autophagolysosomes and reflected their involvement in the pathological process. Demyelination of axons was local in character and was observed around areas with total death of processes, and was most probably secondary in nature, due to activation of fibrous astrocytes. Cells of the oligodendroglia and astroglia were probably not identical in their type of phagocytic activity, for not all of them participated in ingestion of the debris. They may be the explanation of the conflicting data present in the literature regarding the role of the macroglia in immunologic surveillance in the CNS [5]. In the present study and in previous histological investigations of the human brain and the brain of experimentally infected animals, activation of microgliocytes was not observed. Changes in

some of them were more likely to be a manifestation of intracellular edema than of phagocytosis. In AL, as a result of the action of the pathogenic agent, a distinctive type of "paralysis" of the microglia may take place, with the result that they lose their ability to ingest degenerating cells. Incidentally, some investigators also failed to find any evidence of functioning of the microglia as macrophages in other diseases and experimental situations [1, 6]. Experimental AL in guinea pigs is thus a convenient model with which to study both the mechanism of death of the central axons and functioning of the glial cells under conditions when the integrity of the blood-brain barrier is preserved.

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MORPHOLOGICAL CHARACTERISTICS OF HUMAN AND EXPERIMENTAL ANIMAL LEPROMA CELL CULTURES AND EFFECT OF ANTILEPROSY DRUGS ON THEM*

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In view of the absence so far of any reproducible methods for the culture of *Mycobacterium leprae* in cell-free media, the World Health Organization has recognized the need for a search for other approaches to the screening of drugs for antileprosy activity in vitro [8]. The main direction of these investigations is the study of the action of test compounds on various parameters of cells containing *M. leprae* in culture. For this purpose various methods of culture of leproma tissue from human leprosy patients [1, 6, 15] and leproma tissue from animals with experimental leprosy infection [1], infection of cultures of macrophages from blood monocytes of leprosy patients with *M. leprae* [10], cultures of rat and mouse peritoneal macrophages [11] and also of macrophages during culture of nerve tissue, schwannomas, and gliomas [7, 14], have been used. Changes in the quantitative parameters of the dopa reaction [6], the concentrations of thymidine [10, 12] and ATP [9] in the culture, mycobacterial saturation of macrophages [2], and also the ability of *M. leprae*, when subjected to the action of the test compounds, to reproduce in the plantar tissues, were used as criteria of antileprosy activity.

A test system for rapid screening of drugs for antileprosy activity based on their action on a culture of leprous tissue, has been developed at the Research Institute for the Study of Leprosy [2]. In this paper we describe the morphological and functional characteristics of leprous tissue taken from lepromas of varied origin (from patients with leprosy, from experimentally infected nine-banded armadillos and mice) in culture, and the results of their exposure to basic antileprosy drugs.

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