MECHANISM OF ACCUMULATION OF PRION AMYLOID IN THE CNS IN EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS

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UDC 616.831].832-022-036.12-092

KEY WORDS: prions; amyloid; cerebral vessels

Amyotrophic leukospongiosis (AL) is a lethal degenerative nervous disease of man characterized mainly by a lesion of spinal motoneurons, by astrogliosis, and by the development of spongiosis in the white matter of the spinal cord [1]. The etiological agent of AL is related to the agents of Creutzfeld–Jakob disease (CJD) and scrapie, which are members of the group of noncanonical prion viruses. One of the characteristic features of the agents of CJB and scrapie on reproduction in the CNS is their ability to induce the formation of plaques which show signs of both amyloid and concentrations of the prion protein PrP 27-30 kilodaltons [4, 5]. For AL, no studies into virus-induced amyloid production have yet been undertaken.

In this paper prion amyloid was demonstrated in the CNS of guinea pigs infected experimentally with the agent of AL. The dynamics of development of disturbances in the blood-brain and blood-CSF barriers was studied, and structural transformations in the vessels of the CNS and pia mater were examined.

EXPERIMENTAL METHOD

Experiments were carried out on 18 guinea pigs weighing 250-300 g, infected with the agent of amyotrophic leukospongiosis (strain AL-D) by the retrobulbar route [4]. In this method of infection, marked clinical features of the disease (loss of hair, muscular atrophy, development of pareses and paralyses of the limbs and trunk) developed in the 5th-6th week (35th-40th day). Four animals each on the 7th and 14th days after infection (the stage of preclinical manifestations (titer of the agent of AL in the CNS was 3.5-4.1 log ID_{50}/ml), and five animals on the 28th day – the period of initial clinical manifestations (titer of the agent of AL in the CNS was 5-5.6 log ID_{50}/ml) and on the 40th day – the preterminal stage (titer 6-6.2 log ID_{50}/ml) were used for the investigation. The control consisted of nine guinea pigs into which the liquid phase of a 10% suspension of the brain of a clinically healthy person dying from an automobile accident, had been injected.

Electron-Microscopy and Histological Investigations. Under hexobarbital anesthesia the animals were perfused intravitally for 15 min with a mixture of 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). The brain was then removed, pieces of the cerebral cortex, cerebellum, and spinal cord were excised and embedded in paraffin wax for histological study, and in Araldite for ultrastructural analysis by the usual method.

The distribution of prion amyloid was demonstrated histologically by staining with Congo Red by the standard method for polarization microscopy.

Immunocytochemical detection of the agent of AL in the brain sections was carried out in the indirect version of the immunoperoxidase method, using monoclonal antibodies to protein PrP 27-30 of the agent of AL demonstrated in histological sections, on the basis of analysis of the concentration of dark brown complexes. At all times of observation 12-16 sections cut from the same number of specimens were analyzed.

Belorussian Research Institute of Epidemiology and Microbiology, Minsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Votyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 12, pp. 640-643, December, 1991. Original article submitted May 7, 1991.

 0007-4888/91/0012-1802$12.50

TABLE 1. Frequency of Discovery of Concentrations of Prion Amyloid Protein PrP 27-30 in Walls of Blood Vessels in Different Parts of Brain and Pia Mater of Guinea Pigs during Development of Infection (in %)

Test object	Infected animals (time after infection, days)			Uninfected animals (time after injection of uninfected material, days)		
	14	28	40	14	28	40
Cerebrum	2,4±0,15	5,25±0,3**	6,7±0,4*'**	2,2±0,15	Concentrations not discovered	2,6±0,2
Cerebellum	—***	—	5,3±0,3	—		1,6±0,15
Spinal cord	Concentrations not discovered	1,6±0,1**	4,3±0,2*'**	Concentrations not discovered	Concentrations not discovered	1,33±0,15
Pia mater	6,3±0,5*'**	17,2±5,4*'**	76,5±9,1*'**		2.7 ± 0.3	3,25±0,3**

Legend. *) Presence of amyloid-like structures confirmed electron-microscopically; **) presence of amyloid confirmed histochemically (staining with Congo Red); ***) not investigated.

EXPERIMENTAL RESULTS

In histological preparations from animals in the preclinical stage (14 days) and, more clearly, during the period of development of the initial clinical features of the disease on the 28th day, degenerative changes were found in neurons with evidence of astrogliosis and with the formation of cavities of spongiosis in the white matter. No changes were found in the endotheliocytes, pericytes, or basement membrane (BM). Only in the preterminal stage of the disease (40th day) was hypertrophy of the endothelial cells of individual brain vessels and of the pia mater observed.

The histochemical investigation using Congo Red revealed small deposits of congophilic (amyloid) material in the guinea pigs in the preterminal stage of the disease, giving the characteristic color change from red to green, characteristic of amyloid, when examined under the polarization microscope, in the wall of individual small and large vessels (Table 1).

In some preparations (about 15%) larger agglomerations of amyloid were discovered in the form of specific deposits in the walls of the pial arteries (Fig. 1a). The formation of amyloid plaques actually in the tissue of the CNS was not observed.

The immunocytochemical study using antibodies to PrP 27-30 AL revealed the presence of protein PrP 27-30 in the tissue of the CNS as early as on the 7th day of the disease. However, at this time no difference in the staining of the vessel walls compared with the control was observed. Later, some animals tested on the 14th-28th days, and most animals (five of six) tested on the 40th day (Table 1) revealed intense brown staining of small areas of individual vessels in the spinal cord and brain, and in the absolute majority of pial vessels (Fig. 1b). The formation of large antigen–antibody complexes during this period could also be observed outside the vessels, among cells of the pia mater.

The study of animals on the 7th and 14th-28th days after infection, at the ultrastructural level, revealed no change in the vascular bed. By the 28th day, however, intensification of transendothelial metabolism was beginning to be manifested, as a two- to threefold increase in the number of pinocytotic vesicles.

By the 40th day the ultrastructure of the endotheliocytes in 15-17% of vessels showed more marked changes, in the form of translucency of the cytoplasm with destruction of some cytoplasmic organelles, and an increase in the density of the matrix of some mitochondria. Under these circumstances the pinocytotic vesicles were aligned along the plasma membrane facing the lumen of the capillary, or grouped in chains or small agglomerations.

Involvement of a small number of pericytes in the pathological process is noteworthy. Some of them were swollen, and membranous and osmiophilic inclusions accumulated in their cytoplasm, against the background of destruction of the subcellular organelles. Sometimes degeneration in the pericytes was more severe, and amounted to disintegration of all the ultrastructures of the cell, together with nucleolysis. In such capillaries inclusions consisting of agglomerations of amyloid-like filamentous structures 12-14 nm in diameter formed between BM and the endothelium (Fig. 1b).

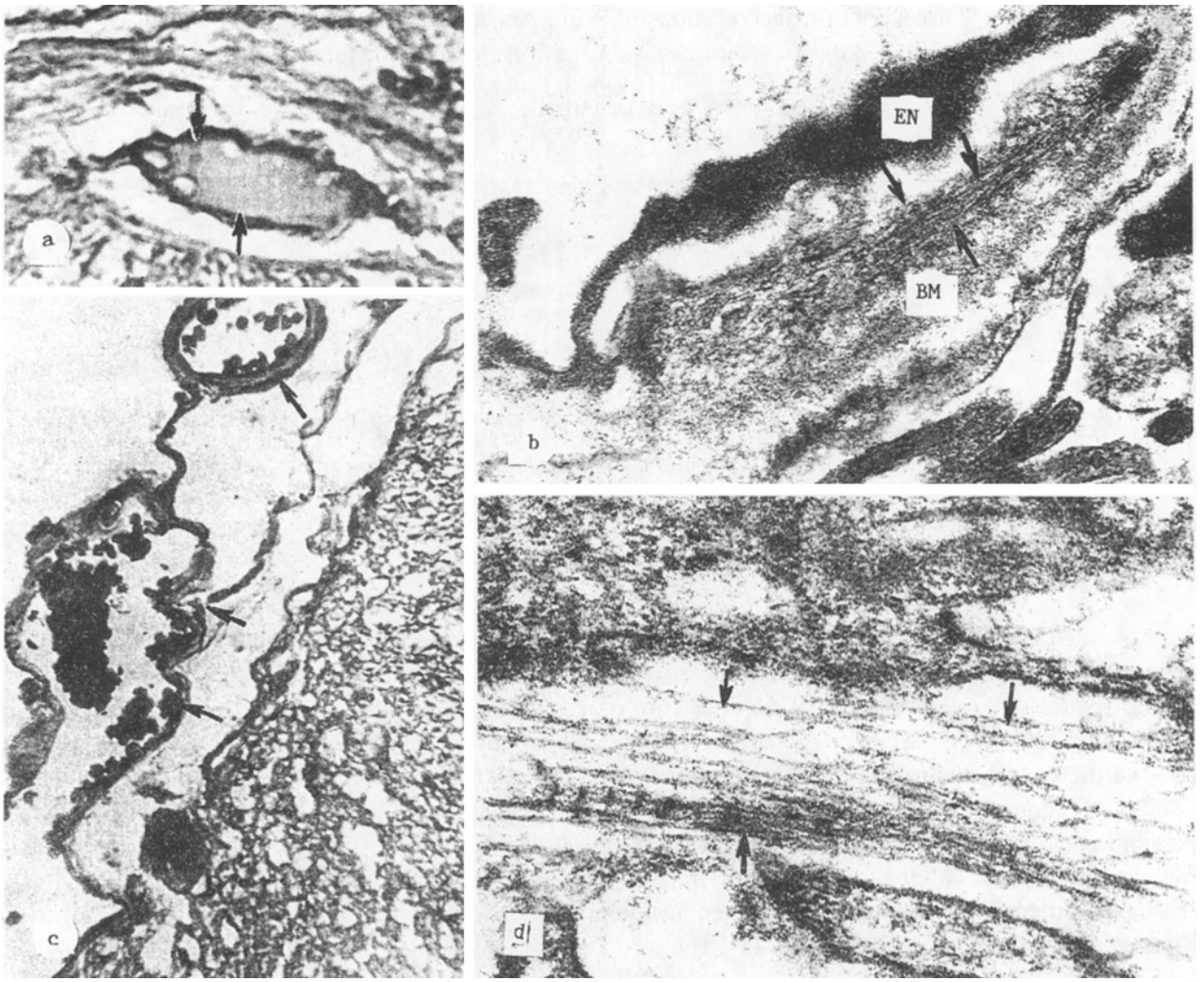

Fig. 1. Guinea pig with experimental AL. a) Cerebral cortex. Agglomerations of antigen of agent of AL in vessel wall (arrows). Indirect immunoperoxidase method using monoclonal antibodies to protein of AL agent PrP 27-30. 700×; b) Pia mater. Deposition of amyloid in wall of vessels (arrows). Stained with Congo Red. 400×; c) Anterior horns of spinal cord, thoracic part. Destruction of pericyte and formation of inclusion (arrow) between endotheliocyte (En) and basement membrane (BM), consisting of filamentous structures. Stained with uranyl acetate and lead citrate. 51,000×; d) Pia mater. Filamentous structures with periodic thickenings (arrows). 82,000×; c, d) ultrastructural changes in capillaries of CNS in guinea pigs with experimental AL.

At the same time activation of macrophages was observed in the pia mater, and collections of abnormal filaments, similar to structures found in the cerebral capillaries, were distributed in the intercellular space among invaginated membranes of squamous epithelium (Fig. 1d). Just as in vessels of the CNS, the latter lay parallel or were chaotically arranged, and attained a length of 1000 nm in the plane of section. Incidentally, the filaments differed morphologically from normal connective-tissue structures.

Previous investigations showed that in experimental AL the development of degenerative changes in the CNS is accompanied by a change in the barrier function of the endothelium and, at the height of the clinical manifestations of the disease, horseradish peroxidase begins to pass through the blood-brain barrier (BBB) [2]. In the present investigation, the earliest structural changes took place in cells of the blood-CSF barrier. Hence it can be concluded that the whole barrier complex is affected in AL.

The agent of AL, the principal component of which is a protein with mol. wt. of 27-30 kD, can be found in the spleen 7-14 days after retrobulbar infection, and it remains there in high concentration throughout the disease. Despite this fact, the agent of AL does not induce any appreciable morphological changes in the spleen cells. The spleen evidently acts as a depot, from which the agent evidently spreads throughout the body via nerve fibers, and penetrates into the CNS through the BBB and blood-CSF barrier. On these grounds, certain new aspects of the pathogenesis of AL can be envisaged. For instance, the virus initially penetrates from the blood into the endotheliocytes, and from them into pericytes which also, like the neuron, are sensitive to the cytodestructive action of the virus. Accumulation in pericytes, the agent of AL causes death of some of these cells. In this case, relatively large deposits of the protein PrP 27-30 are formed in the wall of the blood vessels between BM and endotheliocytes, where it polymerizes and forms prion amyloid filaments 12-14 nm in diameter. The involvement of pericytes, which are now regarded as polypotent mesenchymal cells [3], in the pathological process indicates a disturbance of regeneration in AL. Having passed through the blood-CSF barrier into the CSF the agent of AL evidently reproduces in the lymphocytes of the CSF or in cells of the pia mater of the spinal cord and brain. After multiplication and emerging from these cells, the agent also polymerizes to form agglomerations of filaments in the intercellular space of the pia mater and in the wall of its vessels.

Thus primary involvement of the immune system and hematogenous spread of the virus can be clearly discerned in the pathogenesis of AL. Moreover, the virus enters the CNS most probably not only by retrograde axonal transport, but also through the brain barriers. Experimental AL in guinea pigs can also be used as a model of cerebral amyloidosis of viral nature, in which deposition of prion amyloid in the walls of the CNS and the pia mater is observed.

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