RESPONSE OF ASTROCYTES TO EXPERIMENTAL HERPETIC INFECTION

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The diversity of morphological pictures of the neuroglia and the few investigations that have been undertaken into this problem make it difficult to interpret the significance of changes in glial cells in a pathological process. Of the three different types of cells represented in the CNS, special interest is attached to the study of the astrocytic glia, which is regarded as one type of immunocompetent brain cell [4]. In the writers' previous publication [2] some characteristics of changes in astrocyte structure were examined in experimental herpetic meningo-encephalitis.

The aim of this investigation was to study the structural response of astrocytes to herpetic infection with the aid of a glial marker.

EXPERIMENTAL METHOD

Experiments were carried out on Chinchilla rabbits weighing 2.5 kg. As a model of herpetic infection, 0.02 ml of a suspension of herpes simplex virus (HSV-1, strain L2) was applied in a dose of 3 log $TCPD_{50}$ to a scarified part of the conjunctiva. The glia was discovered in frozen brain sections on the 3rd, 6th, 9th, 12th, and 21st days after infection by the indirect immunoperoxidase method, and monoclonal antibodies to vimentin, a cytoskeletal protein of astrocytes [6], were used. Five animals kept in a separate box, with no infection of any kind, served as the control. Antivimentin antibodies (111D3) are a marker of astrocytes and ependymal cells in the brain [3]. One of a number of serial cryostat sections and also part of the material embedded in paraffin wax were stained with thionine by Nissl's method, and with hematoxylin and eosin. To detect astrocytes, the method of staining with gold and mercuric chloride, described by Cajal, also was used.

EXPERIMENTAL RESULTS

All the infected animals developed a clinical picture of keratoconjunctivitis toward the 3rd day of the experiment, but by the 7th day symptoms of encephalitis could be observed. In the acute stage of the disease (until the 10th day) 20% of the animals died, and in those which remained alive, the infection assumed the chronic form. On the 9th day after infection the titer of virus isolated from the brain substance was 3.5 log $TCPD_{50}$, evidence of the development of an acute infectious viral process.

Immunocytochemical investigation of the brain of the uninfected animals (control) revealed 111D3-positive staining in the limiting glial membrane, around vessels of the microcirculatory bed, in the ependymal cells, and in astrocytes in the large bundles of white matter of the cerebral hemispheres (Fig. 1a, a′).

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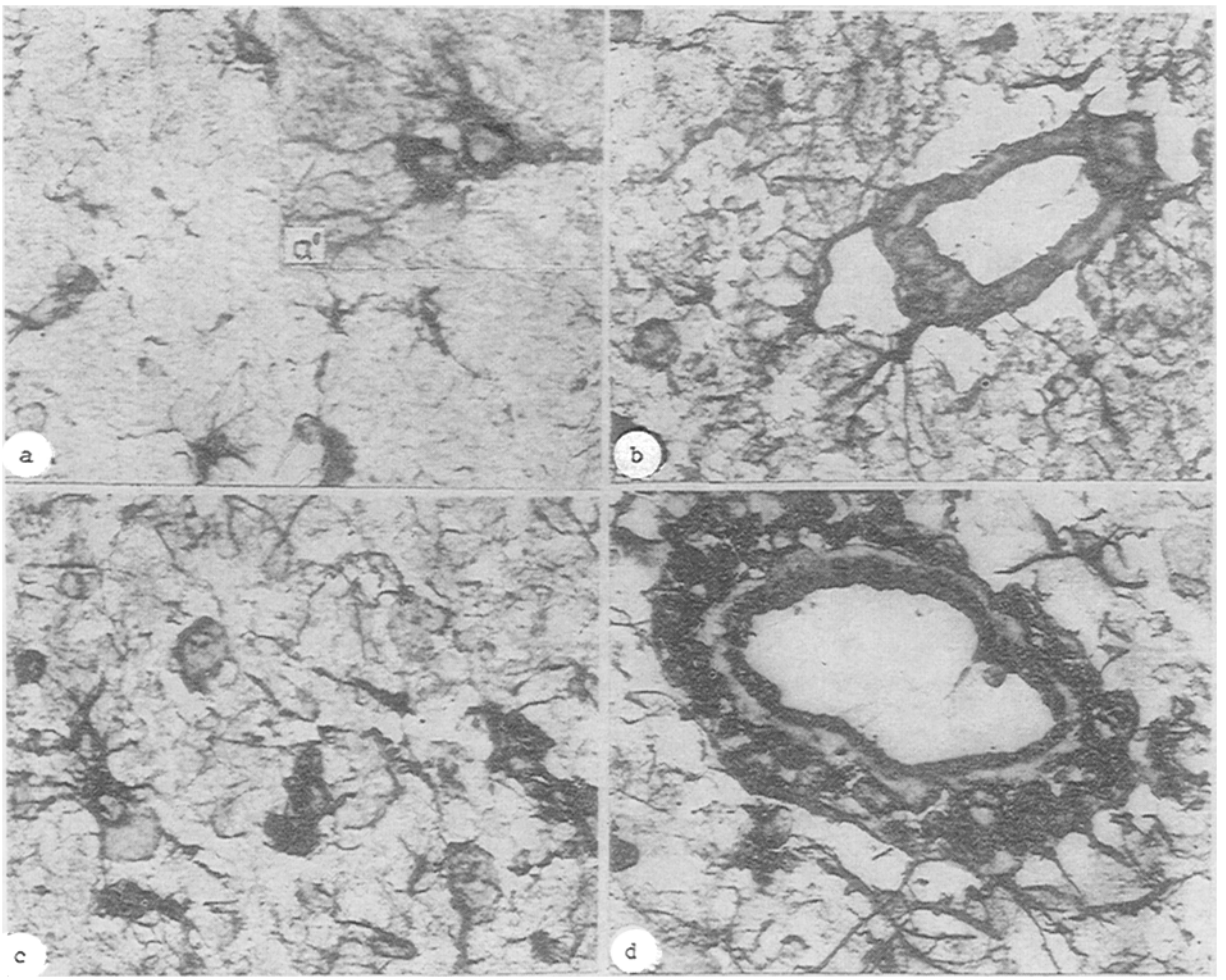

Fig. 1. Reaction of astrocytes in 1st-2nd week of experimental herpetic brain infection: a) astrocytes of large bundles of white matter of brain in uninfected animals (control). 200×; a′) Two astrocytes in white matter of brain under high power (control). Clear outlines of cytoplasm, pale oval nuclei, few outgrowths leaving cell body. 420×; b) Marked swelling of vascular pedicles of astrocytes on 3rd day of experiment. 420×; c) Focal proliferation of astrocytes. Change in shape of cell bodies, narrow rim of cytoplasm, swelling of nuclei of astrocytes on 6th day after infection with HSV. 420×; d) Focal perivascular proliferation of astrocytes on 6th day of experiment. 320×. a, a′, b, c, and d) Immunoperoxidase stain using monoclonal antibodies to vimentin (111D3).

On the 3rd day after infection intensive staining of the vascular pedicles of the astrocytes was observed, and the latter exhibited nodular swelling (Fig. 1b). The number and extent of the visible vessels increased appreciably, due to the reaction with monoclonal antibodies. By the 6th day of the experiment a further increase in the intensity of the glial reaction was observed. For instance, foci of cell proliferation could be seen in which the astrocytes were larger, had acquired an elongated shape with a deep concavity in the middle part, and their cytoplasm appeared in the form of a narrow rim (Fig. 1c). Some astrocytes were at the stage of division. In another group of astrocytes a wider zone of cytoplasm and also an increase in the number of thin and thick processes was observed. The formation of cell clusters consisting of one or two layers of glial cells around blood vessels, and at the site of dying nerve cells, was very characteristic. These formations were found in the upper and lower layers of the cortex. Foci of perivascular proliferation contained large numbers of astrocytes, tightly packed together. Their cytoplasm was intensively stained, and the processes given off by the astrocytes were thickened. The nuclei of the astrocytes were large and mainly oval in shape (Fig. 1d). Meanwhile signs of clasmatodendrosis could be seen. The density of distribution of the astrocytes varied both within the same focus and in separate parts of the brain. During the same period neutrophilic infiltration was observed in the basal zones of the pia mater, reflecting an early stage of the exudative reaction to herpetic

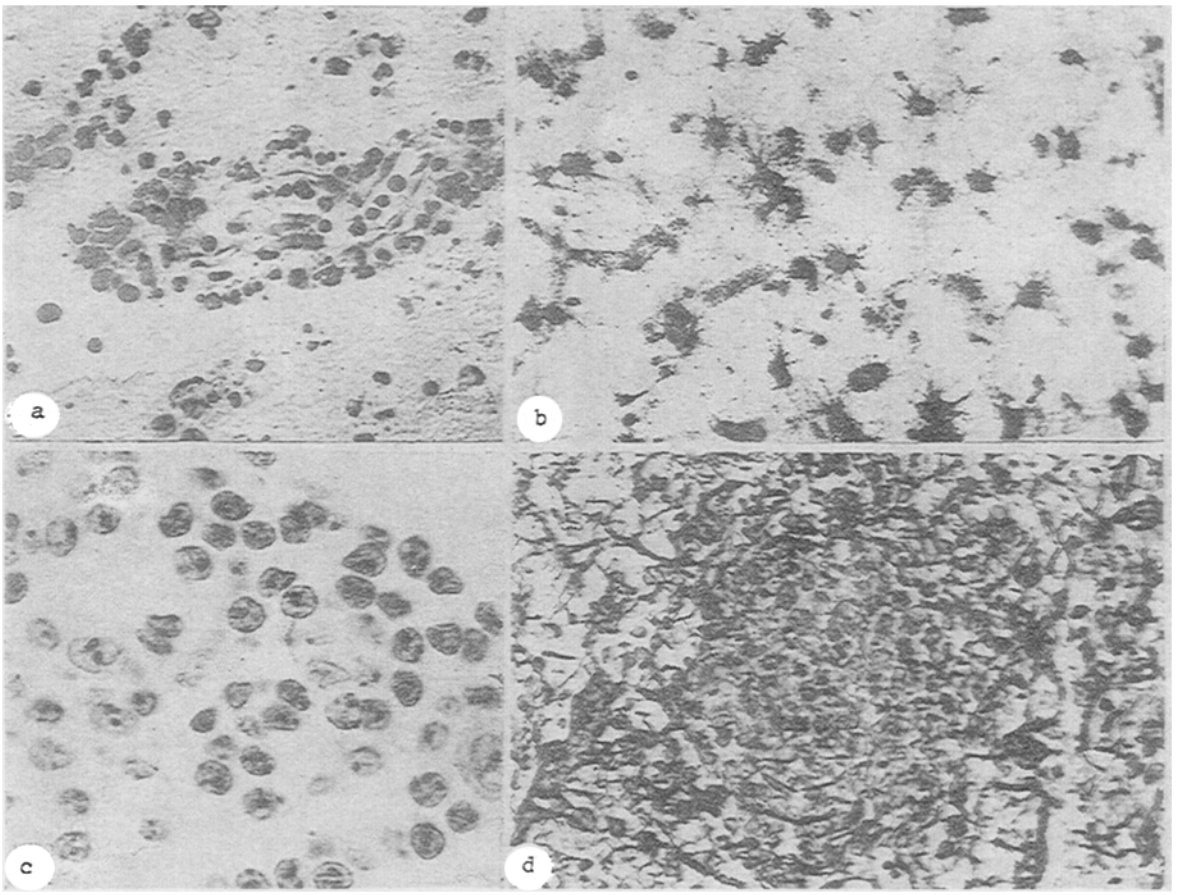

Fig. 2. Changes in astrocytes in 2nd and 3rd weeks of development of herpetic brain infection: a) moderate neutrophilic perivascular infiltration, "severe" changes in neurons in thalamus on 9th day of experiment. Instead of dying cells darkly stained masses and granules are visible. 200×; b) Diffuse proliferation of astrocytes in white matter of cerebral hemispheres on 12th day of experiment. 250×; c) Glial complex of astrocytes in lower layers of cerebral cortex on 12th day of experiment. 320×; d) Glial complex of astrocytes on 21st day of experiment. Obliteration of boundaries between cells is observed in central part of complex. 300×. a, c) Stained with thionine by Nissl's method; b) stained by Cajal's method; d) immunoperoxidase stain using monoclonal antibodies to vimentin (111D3).

infection. Foci of inflammation of the cells were seen in the cerebral cortex. Later the abundance of the destructive changes increased. For instance, on the 9th day of the experiment massive foci of necrosis were formed in the parietal, temporal, frontal, and to a lesser degree, in the occipital lobes. In severely damaged nerve cells chromatolysis, sometimes total, with pycnosis of the nucleus were observed. The outlines of the cell bodies became eroded and blurred. Instead of dying cells, darkly stained masses and granules could be seen. The astrocytes also underwent destructive changes. Inflammatory infiltration of the brain substance at this time was not uniform in different parts of the brain. In the subcortical zones, for instance, it affected a moderate number of neutrophils with an admixture of lymphocytes (Fig. 2a). No inflammatory infiltration could be found in any part of the brain 12 days after infection. Proliferation of astrocytes in the pathological foci dominated the foreground (Fig. 2b). Here also the formation of glial complexes, whose cells had dark, intensively stained cytoplasm and a large pale nucleus, could be observed; the processes of the astrocytes were visible for a short distance. Denser distribution of astrocytes was observed in the central zone than at the periphery of the glial complex. On staining by Nissl's method many of the astrocyte nuclei

appeared dark and they were mainly oval in shape (Fig. 2c). On the 21st day of the experiment further replacement of the tissue defects of the brain by proliferating astrocytes was observed. Unlike at the previous time, however, at this stage the structure of the glial complexes was different. For instance, in the central part of the complex the astrocytes were connected together by cytoplasmic bridges, and features of the cell structure were obliterated (Fig. 2d). Proliferating astrocytes created a picture of anisomorphic gliosis. These foci stained less intensively than the glial complexes in the acute stage of the infectious process, and they lacked clear boundaries with surrounding tissue.

Thus the use of a specific marker, namely monoclonal antiastrocytic antibodies, revealed that the earliest structural reaction in the brain to herpetic infection consists of changes in the astrocytic glia. Progressive changes in astrocytes [1] during the infectious process are "wavelike" in character. For instance, activation of astrocytes preceded alteration of the brain substance, it intensifies with an increase in destructive changes in the nerve cells. In the period of neutrophilic infiltration of the brain substance, however, no proliferative response of the astrocytes is found, evidently because of its inhibition during this period. Later during proliferation of astrocytes with the formation of glial complexes once again it becomes a mandatory sign of morphological changes in the brain of the infected animals.

The use of an immunohistochemical method shows that the response of the astrocytic glia varies at different times of the infectious process. It can be tentatively suggested that at each stage there is a reaction of astrocytes of different subtypes. An understanding of the particular features of the structural response of the astrocytes is very important. According to Roessmann and Gambetti [5], diffuse proliferation of astrocytes is an indicator of considerable brain damage, in agreement with the results of our own experimental investigations.

The dynamics of the morphological changes in the astrocytic glia in the course of the virus disease is a reflection of the functions of these cells, namely barrier, eliminative, and replacement. In our view, this character of the structural response of the astrocytes coincides with, and also evidently is linked with the secretion of interferon, interleukin, and other mediators by these cells [7], so that it is possible that these cells may have a definite modulating effect on the development of information in the brain.

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