Accumulation of Factor Stimulating Glial Proliferation in Aging Mammalian Brain

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Extract from the brain of young mice produced weak cytoproliferative effect on cultured glial cells, while brain extract from old mice 3-4- and 30-60-fold stimulated glial proliferation in primary and passaged cell cultures, respectively.

Key Words: *cell culture; gliosis; brain aging; prion diseases*

Human and animal prion diseases (PD) are characterized by typical histopathological changes involving only the brain and, sometimes, spinal cord. They are characterized by neuronal loss, spongiform changes of the white and/or grey matter of the brain and spinal cord, formation of amyloid plagues, and gliosis. These changes considerably differ in various PD. Thus, amyloid plagues are observed in 9 and 70% patients with Creutzfeldt-Jacob disease (CJD) and kuru, respectively. However, gliosis is typical of all diseases including lethal hereditary insomnia, which is seldom characterized by spongiosis, but induces neuronal loss and astrogliosis in the mediodorsal and anterioventral thalamic nuclei [10].

For a long time much attention was focused on the mechanism of PD-induced lesions in the central nervous system (CNS), plague origin, chemical composition, and topography, as well as topography of spongiform regions, mechanisms of neurodegeneration, and correlations between CNS damage and mutations in PRNP gene. At the same time, it was assumed that the initial histopathological changes are neuronal loss followed by spongiosis and accumulation of amyloid. This sequence culminates in glial reaction replacing defects (reactive gliosis) caused by progressive neuronal death [1].

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Recently, the interest of many researchers was attracted to the role of glia in this multistage process of CNS damage by infectious (and other) proteins. In the late 80s, immunocytochemical and ultrastructural studies showed that, on one hand, glial cells are involved in phagocytosis of amyloid fibrils during Alzheimer disease (AD) [11,15], while on the other hand, they participate in the formation of amyloid fibrils during AD and CJD [9,14]. These data were confirmed by the discovery of activated microglia involved in the accumulation of amyloid during experimental scrapie in mice [2]. At the same time, variable and important role of microglia in the formation of amyloid plagues during PD was demonstrated. It was suggested that variability is specific for this nosologic form and realized on the level of mRNA synthesis for scrapie-associated amyloid protein precursor [7].

These data were supplemented by studies on the mechanisms of neuronal death. It was shown that synthetic peptide 106-126 homologous to amyloid protein isolated from the brain of patient died from Gerstmann—Straussler—Scheinker syndrome (GSSS) caused apoptotic death of neurons and pronounced proliferation of glial cells in culture [13]. Moreover, it was found that peptide 106-126 promotes *in vitro* formation of amyloid fibrils and is toxic for cultured neurons only in the presence of microglia responding to this peptide by enhanced generation of oxidative radicals [3].

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Direct analysis of the sequence of events in the brain of scrapie mice showed that glial activation and cytokine immunoreactivity develop much earlier than spongiosis. Neuronal apoptosis is preceded by cytokine production by activated glia [13]. Similar events were described during AD characterized by immunoreactive effect of glia on interleikins 1 and 6 and the formation of amyloid plagues [13]. Therefore, it was assumed that neuronal loss during scrapie and AD is mediated by the products of activated glia [4,8].

Another original approach also confirmed the important role of glia in prion pathology. Thus, amphotericin B or its less toxic derivative increased the survival of scrapie mice and inhibited accumulation of infectious prion protein (PrP^{Sc}) in the brain and hyperexpression of astrocyte-specific glial fibrillary acidic protein [13].

The similarity between brain disorders during aging and PD-induced changes was first noted about 30 years ago. It was shown that the brain in aging mice is characterized by neuronal loss, glial proliferation, and the formation of amyloid plagues. Soon, similar changes were found in the brain of old dogs and monkeys. Thereafter, pathohistological changes typical of kuru, CJD, and GSSS were revealed in people with AD, senile dementia, and even in healthy elderly people [12].

Similarity of pathologic changes in the brain during aging and PD confirm the important role of glia in these states. Moreover, amyloid precursor in AD, normal aging, and Down syndrome is encoded by the same gene, which is highly conservative and mapped [6] as well as prion gene.

Thus, we assumed that some glial stimulating factor(s) could accumulate in the brain of aging mammals including humans, which could be regarded as an important component in the morphogenesis of these changes including neuronal dystrophy. This can result in neuronal loss and, finally, in degeneration of the whole brain.

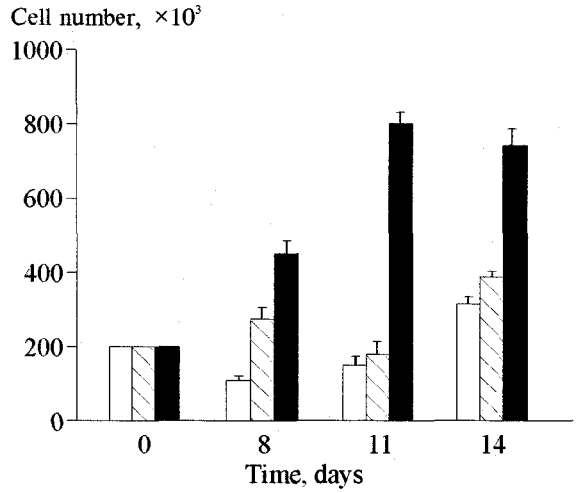

Fig. 1. Stimulation of glial proliferation in primary cultures of the mouse brain with brain extracts from young and old mice. Here and in Fig. 2 and 3: open bars: control; hatched bars: extract from young brain; dark bars: extract from old brain.

In order to test this assumption we analysed the possibility to stimulate glial proliferation in culture with a factor accumulated in aging mammalian brain.

MATERIALS AND METHODS

Minced brain from 3-5-day-old C57Bl/6 mice was incubated in 0.25% trypsin for 10 min at 37°C, centrifuged, washed 3 times with Eagle's DMEM, and pipetted in culture medium (Eagle's DMEM, 3% glutamine, 10% fetal calf serum, 1% HEPES, antibiotics).

Glial cell monolayer (10 days *in vitro*) was passaged routinely using 0.2% versen. Initial cell density in the suspension was 200,000 cells/ml. Dissociated

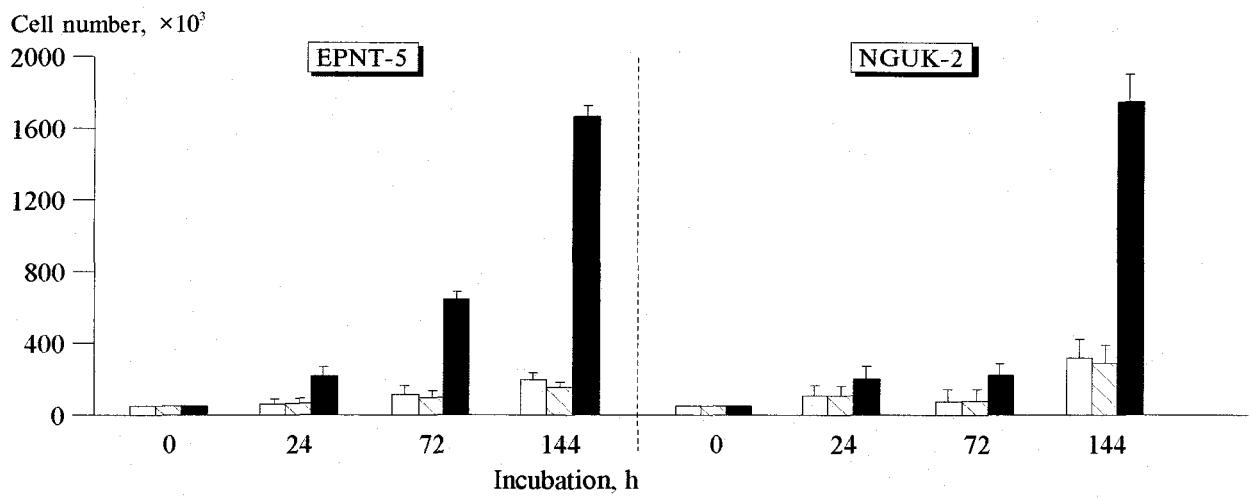

Fig. 2. Stimulatory effect of brain extracts from young and old mice on proliferation of EPNT-5 and NGUK-2 cell.

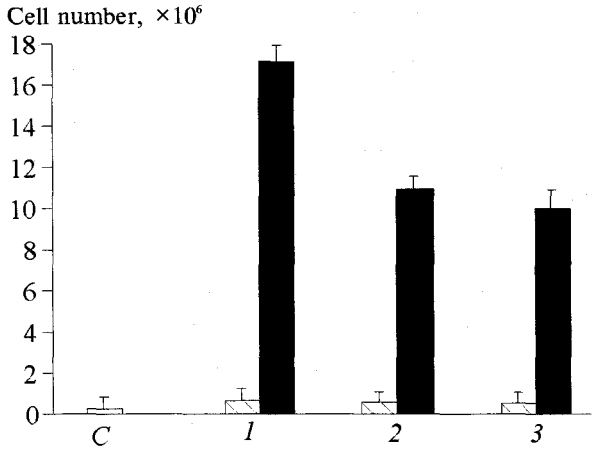

Fig. 3. Stimulatory effect of brain extracts from young and old mice on proliferation of L_{929} cells on day 3 of culturing. C: control; incubation with brain extract diluted 1:200 (*1*), 1:500 (*2*), and 1:2000 (*3*).

glial cells were cultured in Leiton tubes on glass coverslips, in the same medium in the presence of sterile mouse brain extract. Cells were detached from the coverslips with 0.2% versen and counted in a Goryaev chamber.

The following cell lines were used: EPNT-5 (mouse ependymoblastoma), NGUK-2 (neurinoma of rat Gasserian ganglion) and L_{929} (murine embryonic lung). The cells were cultured in the same medium by routine technique.

Brain extracts (25%) were prepared from the brain of young (2 months) and old (1.5 years) mice by homogenization in a porcelain mortar with synthetic corundum and $^3/_4$ culture medium. The suspension was centrifuged for 10 min at 3000 rpm. The supernatant was filtered through Millipor filters (0.45 μ). The results were processed statistically with Student's *t* test.

RESULTS

In experimental series I we examined the effect of brain extracts obtained from mice of different age and diluted 1:50 on proliferation of primary trypsinized glial cells. To this end, in each experiment all tubes with cultures were divided into 3 groups: control (1), cultures containing extract from the young (2) or old (3) brain.

Extracts from young brain weakly (less than 2-fold) stimulated glial proliferation only on day 14 of observation. Extract from old brain 2-fold stimulated glial proliferation as soon as on day 8 and 3-4-fold by days 11-14 (Fig. 1).

The observed stimulatory effect of the extract from old mouse brain on proliferation of primary glial cells prompted us to examine this effect on cultured cells of various origin, in particular, neural EPTM-5 and NGUK-2 cells (Fig. 2).

Extract from young brain had no stimulatory effect on cell proliferation in both lines: after 144 h incubation the number of cells in culture did not exceed or even was below the control.

Extract from old brain produced dramatic stimulatory effect. In EPTM-5 and NGUK-2 cultures cell counts increased 8.3- and 5.5-fold, respectively, compared to the control and 33- and 35-fold compared to the initial values.

The experiments with L_{929} cells showed that extract from young brain diluted 1:2000, 1:500 and 1:200 stimulated cell proliferation no more than 2-2.3-fold. The same dilutions of the extract from old mouse brain enhanced cell proliferation 61-, 39-, and 36-fold, respectively, as compared to control.

These findings confirmed accumulation of a factor stimulating glial proliferation in aging mouse brain. We cannot speculate on the nature of this factor, because accumulation of some viral agent in aging brain also cannot be excluded. However, experiments with L_{929} cells showed that this factor possesses universal cytoproliferative activity, because it was most pronounced in somatic cells. These data can serve as a basis for further investigations, in particular, fractionation of brain extracts and purification of the factor, which could contribute to the understanding of its nature and serve as a basis for further experiments on animals.

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REFERENCES

1. V. A. Zuev, I. A. Zavalishin, and V. M. Roikhel', *Prion Diseases of Humans and Animals*, Moscow (1999).
2. E. Beck and P. M. Daniel, in: *Prions*, Eds. S. Prusiner and M. P. McKinley, New York (1987), pp. 331-385.
3. D. R. Brown, B. Schmidt, and H. A. Kretzschmar, *Nature*, **380**, 345-347 (1996).
4. M. E. Bruce, P. A. McBridge, M. Jeffrey, *et al.*, in: *Alzheimer's Disease: Advances in Clinical and Basic Research*, New York (1993), pp. 481-487.
5. H. Fraser and P. A. McBridge, in: *Senile Dementia of the Alzheimer Type*, Eds. J. Traber and W. H. Gispen, Berlin (1985), pp. 250-268.
6. D. Goldgaber, M. I. Lerman, W. O. VcBride, *et al., J. Neural Transm.*, **24**, 23-28 (1987).
7. D. C. Guiroy, I. Wakayama, P. Liberski, *et al., Acta Neuropathol.* (Berl.), **87**, 447-450 (1994).
8. P. Eikelenboom, S. S. Zhan, W. A. Wan Gool, *et al., Trends Neurosci.*, **15**, 447-450 (1994).
9. K. Hikita, J. Tateischi, and H. Nagara, *Acta Neuropathol.* (Berl.), **68**, 138-144 (1985).
10. J. Ironside and J. Bell, in: *Prion Diseases*, Eds. J. Collinge and M. Halmer, Oxford (1997), pp. 57-88.

11. S. Itagaki, P. L. McGeer, H. Akiyama, *et al., J. Neuroimmunol.*, **2**, 173-182 (1989).
12. C. L. Masters, G. Malthaup, G. Simms, *et al., EMBO J.*, **4**, 2757-2763 (1985).
13. *Transmissible Subacute Spongiform Enchephalopathies*, Eds. L. Court and B. Dodet, Paris (1996).
14. H. M. Wisniewski, A. W. Verbrodt, R. C. Moretz, *et al., Exp. Brain Res.*, **5**, Suppl., 3-9 (1982).
15. H. M. Wisniewski, M. Barcikowska, and E. Kida, *Acta Neuropathol.* (Berl.), **81**, 588-590 (1991).