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Prion Neurodegeneration as Factor for Increasing of Astrocytosis and Microgliosis in CNS

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In order to investigate the cellular basis of gliosis accompanying prion pathology we have selected four zones in mammalian CNS (cortex, thalamus, hippocampus and cerebellum) providing a relatively simple access to the most affected by degeneration brain areas. The intensification of the cellular reactivity of brain cells is an important pathomorphological feature *in situ* contributing for prion disease diagnosis as neuronal loss and spongiosis. Immunohistochemical approaches using specific monoclonal antibodies V9 and 5D4 was performed. The examination of hamster brains affected by experimental scrapie (263K agent strain) was accomplished to determine the extent of the astrocytosis and microgliosis during degeneration. New non-conventional methods for visualizing of the glial reaction in CNS were applied.

Key words: central nervous system (CNS), prion neurodegeneration, astrocytosis, microgliosis.

Introduction

Prion neurodegeneration is result of transmissible, sporadic or hereditary conditions characterized by progressive nervous system dysfunction. Fatal prion diseases or transmissible spongiform encephalopaties (TSE) affect humans (Creutzfeldt-Jacob disease variants, fatal familial insomnia, Gerstmann-Straussler-Scheinker syndrome) and numerous other mammalian species ("mad cow" disease, scrapie, chronic wasting disease, spongiform encephalopathies in cat family etc.). In the course of prion diseases a long incubation period is followed by histopathological changes restricted in CNS. General histopathological signs in CNS during prion degeneration are spongiosis, gliosis or reaction of resident glia, neuronal death, appearance and concentration of pathologic isoform of prion protein (PrPres), and specific amyloid plaque formation.

The cellular reactivity in degenerating brain is registered by morphological methods as a decreasing (for neuronal population) or an increasing (for astroglia and microglia) in the number and density of the brain cells. Glial reaction during prion neurodegeneration has two different basic components: astrocytosis and microgliosis, united in the common neuromorphological phenomenon — *gliosis*.

Recently introduced in prion biology the vimentin immunoreactivity for astrocytes [5, 8] and keratan sulfate immunohistochemistry for ramified microglia [3] were used in this study for evaluation of the extent of gliosis and glial cell density in four brain levels during development of the experimental prion disease.

Material and Methods

Infection of animals: Adult 45-week-old female outbred golden Syrian hamsters were injected intracerebrally into the right hemisphere with 50 µl of 1% w/v brain homogenate from 263K scrapie strain (2.2×10^{11} 50% lethal dose/g). Anaesthetized hamsters (injected and controls) were sacrificed at 30^{th}, 70^{th} and 87^{th} days after agent inoculation (incubation period, florid and terminal stages of the prion encephalopathy). The brains were fixed in Carnoy's solution at room temperature overnight. Serial transversal sections 5-7 microns thick were obtained from selected levels using Leica paraffin microtome after embedding in paraffin.

Immunohistochemical localization of the astroglial reaction in CNS: Slides with transversal brain sections from selected regions (5-7 microns thick) were blocked for endogenous peroxidase (H_2O_2/methanol 1:60) and for non-specific IgG tissue binding (20% normal horse serum). Incubation with anti-vimentin monoclonal antibodies (Dako) clone V9 diluted 1:5000 for 24 hours at 4°C was performed the first day. As secondary antibody was used biotinilated anti mouse IgG (1:500 diluted) followed by application of ABC (Vector) kit and diaminobenzidine (DAB) substrate. Controls: whole immunostaining procedure without anti-vimentin antibodies and whole procedure using nonspecific second antibodies.

Visualization of the microglial reaction in CNS by keratan sulfate immunohistochemistry: Commercially available anti-keratan sulfate monoclonal antibodies 5D4 (Calbiochem-San Diego CA), clone5-D-4, isotype IgG1, k, were used for immunohistochemical procedure. Optimal working dilution of monoclonal antibodies was precised as 1:1000 for overnight incubation at 4°C. The next day a procedure, using PicTure Polymer Detection System — a horseradish peroxidase/Fab fragment polymer conjugate (ZYMED), was applied for 45 min at room temperature. DAB substrate kit for peroxidase (VECTOR) was used as diaminobenzidine chromogen source for 5-10 min. Controls: Whole immunostaining procedure without 5D4 mAb and whole procedure with diluted 1:1000 5D4 mAb in PBS containing 100 mg/ml type I and II keratan sulfate in the solution.

Studied CNS regions: cerebral cortex, hippocampus, cerebellum and thalamus.

Results

Immunodensitometric assay for vimentin immunoreactivity was made in the 4 selected regions of the affected by the prion neurodegeneration hamster CNS. In general, astrogliosis demonstrated by the vimentin re-expression during prion pathology is easier to estimate because vim (+) astroglia lacks in adult healthy brain (absolutely negative control!). During the earlier periods of development of prion degenerative disease (florid stage) astrogliosis estimated by the appearance of vim (+) cells is less intensive. It's important to note that during the florid stage of the prion disease scrapie 263K there is regional distribution of the groups of vim (+) astroglia in small foci of gliosis (Fig.1). The distribution of the vim (+) astrocytes in this period could

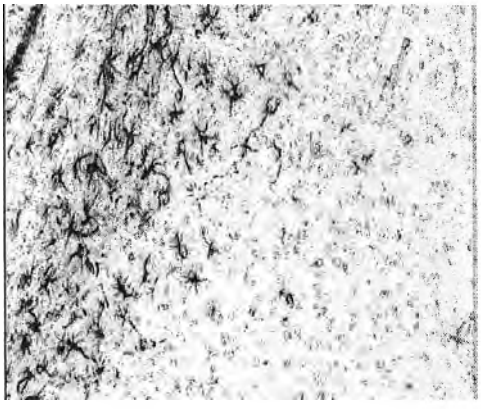

Fig. 1. Astrocytosis - regional distribution of the concentrated in small foci vim (+) astroglia. Thalamic area of affected by scrapie 263K hamster brain. Vimentin immunohistochemistry with *mAb V9.* × 100

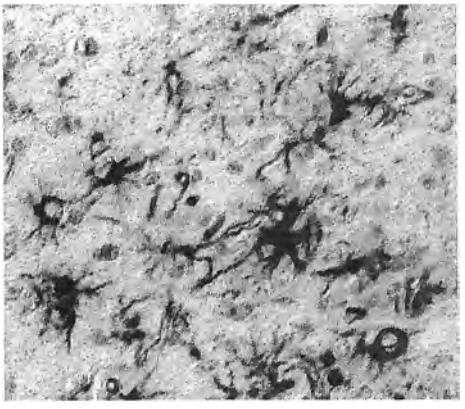

Fig. 2. Large areas of confluent vimentin positive cells during terminal stage of the prion neurodegeneration in hamster brain. × 480

be registered in thalamus, hippocampus and cortex. At 70th day post inoculation (dpi) of the scrapie agent many vim (+) cells were located in deep cortex layers but they were at the same time less prominent in medium layers. Astrocytic morphology of these cells is most evident especially in the cortex ant thalamus. There they had morphological traits of mature hypertrophic astrocytes. Between 70th and 87th dpi vimentin immunoreactivity in thalamus and hippocampus demonstrates 3-5-fold increase forming large areas of positive cells (Fig. 2). Starting from lacking of vim (+) cells in pre-clinic incubation stage (30th day after infection) a progressive increase of astrocytic reaction measured by vimentin immunoreactivity was demonstrated in studied brain areas.

Keratan sulfate immunohistochemical (KS-IHC) visualization of microglial reaction during prion degeneration is more demonstrative in comparison with conventionally used till now lectin methods. 5D4 (+) cells in hamster brain are abundant in cerebral cortex, hippocampus, thalamus and cerebellum of the scrapie-infected hamster brain. The course of the prion neurodegeneration checked by this microglial reactivity in 30th, 70th and 87th dpi changes microglial density in these regions (Fig. 3). A non-essential decrease of the cell density is registered during incubation stage (30th dpi). In the florid (70th day) and terminal (86th day after infection) disease stages we found an essential increase of the density of ramified type 5D4 (+) microglia in the cortex, thalamus, hippocampus and granular layer of the cerebellum. The most of the 5D4 (+) cells have big cell shapes respectively its branching morphology, numerous cytoplasmatic processes and ramified structure. The nuclei are prolonged and relatively small. The cytoplasm is moderately marked by the reaction product in contrast of the strongly positive to keratin sulfate nucleus. Microgliosis during degenerative changes in CNS (estimated by KS-IHC) is co-localized with another histhopathological sign of degeneration — spongiosis (Fig. 4).

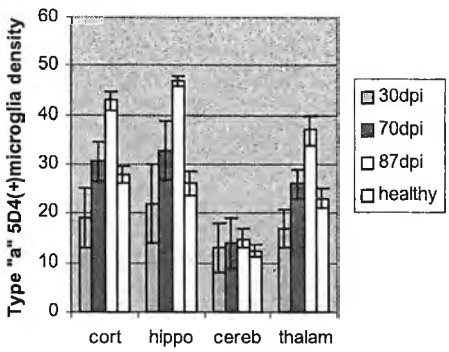

Fig. 3. Profiles of the 5D4(+) microglial reactivity during the course of prion neurodegeneration

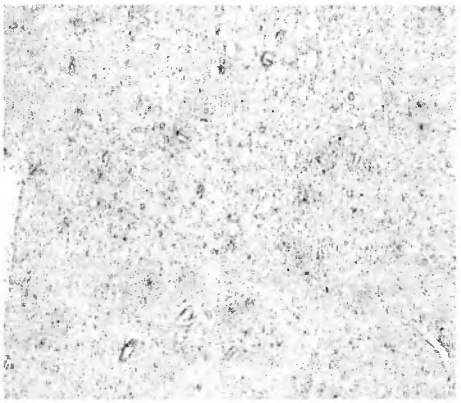

Fig. 4. Parallelism of the cellular reactivity (microgliosis) and spongiosis in the periventricular area of the hamster brain. Terminal stage of experimental scrapie 263K in hamster. Keratan-sulfate immunohistochemistry. × 80

Discussion

Astrogliosis, registered as re-expression *de novo* of vimentin marker in mature CNS during prion neurodegeneration is an additional biological marker of the pathogenesis of transmissible spongiform encephalopathies. This marker is useful not only for diagnostics but for the studies onto neurodegenerative changes. In morphological diagnostics of the prion degenerative diseases now currently is in use simple immunohistochemical detection of GFAP (+) astroglia. We propose and use in this work vimentin as additional marker for demonstration of the gliosis in prion infected brains. Vimentin immunohistochemistry possesses different characteristics and advantages revealing astrogliosis as one of the more important histopathological sign of prion degeneration. The appearance of vim (+) cells does not result from the insult made by intracerebral trauma during agent inoculation suggested earlier [4]. In the prove of this is the fact that in control animals injected with non infected brain vim (+) cells were observed rarely only in the right side of brain around the needle tract.

Microglial reaction, or microgliosis as a regional or local increasing of the microglial concentration, is described in parallel with degenerative changes in prion diseases. A correlation between prion deposition, spongiform degeneration and proliferation of the reactive microglia (microgliosis) is found in some CNS regions — thalamus, pons, cortex etc. [9, 10]. Our present results show a stable correlation between the morphological signs of spongiform degeneration and the relative increasing of reactivity of the 5D4 (+) subgroup of the microglia. Studies by J a n d e r et al. [7] confirm at minimum a large preservation of the 5D4 expression in degenerative lesions in contrast of the lacking of the T-cell-mediated autoimmune inflammation. Microglial activation results brain damage and triggering of the neurodegenerative process in [6]. According to B e r t o l o t t o et al. [2] there is no correlation between cellular activation and expression of the keratan-sulfate epitope from the 5D4 (+) microglia. A possible explanation of the microglial reaction during prion neurodegeneration could be the presumption that microglia can participate in the prion agent clearance and at the same time they act as reservoir [1].

In this study the wider distribution as well as higher density of 5D4(+) microglia and vim(+) astrocytes have been demonstrated with two newly introduced procedures. Immunohistochemistry for keratan sulfate and vimentin epitopes in scrapie-affected CNS is useful additional tool in diagnostics and for morphological approaches of the prion neurodegeneration.

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