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Complement Factors C1q and C3b in Brains with Creutzfeldt-Jakob Disease*

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Neuronal loss in Creutzfeldt-Jakob disease (CJD) is due to apoptosis and autophagy, however the apoptotic cell death may be related to the influence of cytokines. The role of complement system in induction of this process was recently postulated. In our study we examined the immunoreactivity for "early activation" complement factors C1q and C3b in the brains of the patients with CJD. We showed the positive immunoreactivity for C1q and C3b in neurons mainly in areas of spongiform change, in Purkinje cells, molecular layer and granular layer of cerebellum, and in prion protein positive plaques. The evident positive reactivity of two complement factors may suggest the role of complement system in neurodegeneration in CJD.

Introduction

Creutzfeldt-Jakob disease (CJD) is a progressive neurodegenerative disease classified among prion diseases, or transmissible spongiform encephalopathies (TSEs).

Like other human prion diseases CJD is characterized neuropathologically by spongiform change, neuronal loss, gliosis and accumulation of pathological isoform of prion protein PrP^{sc} [1].

Neuronal loss is caused by apoptosis. However, apoptotic cell death may be triggered by cytokines released from astrocytes and microglia [5, 10, 13].

The role of complement activation in neurodegenerative disorders has been shown by some investigators, and its role in prion disorders was recently postulated [8].

The complement system consists of some 30 fluid-phase and cell membrane proteins responsible not only for the recognition of diverse pathogens, but also for killing microbial agents like bacteria, viruses or parasites, while preserving normal host cells [2].

The central nervous system (CNS) is separated from the blood by the blood-brain barrier (BBB), so the passage of large molecules and immunocompetent cells from plasma to brain is restricted [11].

The CNS may participate in the inflammatory response by an intrinsic production of cytokines. Some chronic neurological disorders were recently reclassified as "neuroinflammations" – that are CNS specific, inflammation-like glial responses, which may engender neurodegenerative events including plaque formation, dystrophic neurite growth, or hyperphosphorylation of MAP-tau [13].

Rapid advances in neurobiology led to a recognition that glia may respond to tissue insult with a complex array of pro-inflammatory cytokines. Microglia are now recognized as the prime components of this intrinsic brain immune system [13].

Furthermore, microglia, neurons and oligodendrocytes may produce complement factors [3, 6].

Primary cultures or different cell lines of human origin were used to show that glial cells and neurons *in vitro* were capable of producing almost all complement proteins, particularly following the stimulation with cytokines [3].

Furthermore, complement may play same role in TSEs. Mice deficient in C3, C1q and Bf/C2 were partially or fully protected against scrapie upon intraperitoneal exposure [7].

The aim of our study was to investigate immunoreactivity of CNS cells for complement factors C1q and C3b in brains of patients died of CJD.

Material and Methods

The samples of brain and cerebellum were obtained from CJD brain autopsies. The final diagnosis was estab-

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lished based on the presence of neuropathological findings and PrP^{sc} immunoreactivity [1]. The tissue samples were taken from temporal and frontal lobes of the brain and from vermis of the cerebellum.

Tissue material was fixed with 9% buffered formalin and washed in formic acid, than routinely processed to paraffin and stained with HE. Routine neuropathological examination was performed and typical changes for CJD were present.

Additionally, the typical plaques were found by PrP immunohistochemistry with anti-PrP antibodies obtained from Chemicon following the procedure proposed by the producer.

To visualize the activity of complement factors C1q and C3b the appropriate antibodies from DAKOCytomation were used.

The brain tissue samples from healthy individuals died of other than neurological reasons were used as controls.

Results

We found the positive reaction of C1q in neurons of molecular and granular layer of the cerebellum. Positive reaction was evident and very strong in Purkinje cells. PrP-positive plaques showed high positive reactivity. Both astroglia and microglia were also positive for C1q.

Several neurons were strongly positive in the areas of spongiform change.

C3b immunoreactivity was found in plaques, neurons of granular and molecular layer, and Purkinje cells of the cerebellum, and in neurons of the brain cortex. Astrocytic reactivity was strong.

Controlled brains showed no complement immunoreactivity.

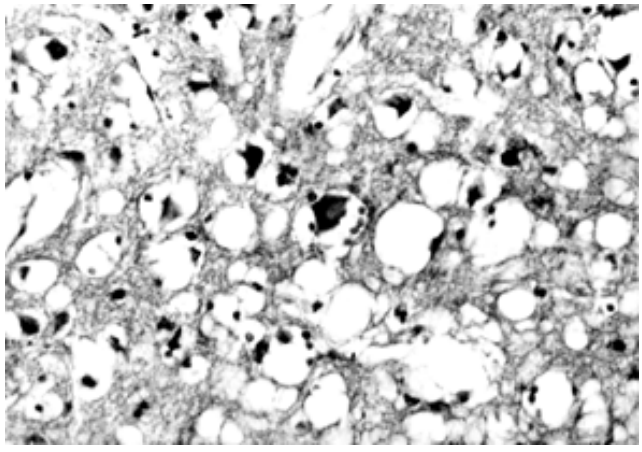

Fig. 1. C3b-positive neurons in area of evident spongiosis, anti-C3b antibody DakoCytomation, Magn. 400×.

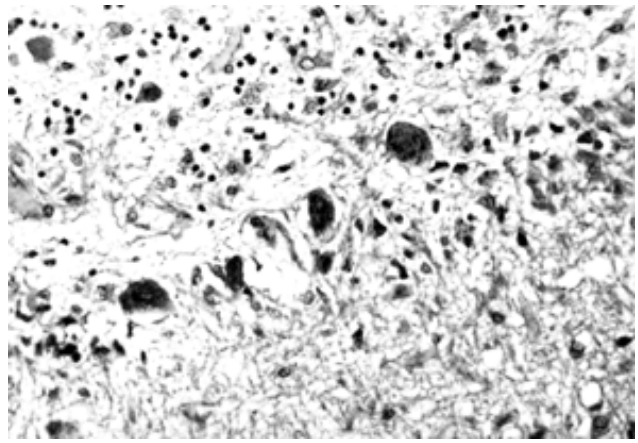

Fig. 2. Purkinje cells, C1q-positive, anti-C1q antibody DakoCytomation. Magn. 400×.

Discussion

Increased focal biosynthesis of complement and uncontrolled complement activation in the CNS are contributing factors in pathology of degenerative disorders leading to neuronal loss and focal inflammation [11].

Complement may be activated by two distinct pathways, on the classical or the alternative pathway. Interaction of C1q with antibody-antigen complexes or interaction of C1q with non-immune molecules (like serum amyloid P) may activate a classic pathway [4].

Initiation of the alternative pathway does not depend on the presence of immune complexes and leads to a deposition of C3 fragments: C3b and C3b on the target cells [12].

The brain cells may express complement and regulatory proteins of the complement cascade particularly in response to an infectious challenge [3, 14].

Endogenous source of cytokines raises the possibility of complement activation and complement-mediated neurotoxicity in the brain without BBB breakdown. The balance between resistance to complement and susceptibility is dependent on complement inhibitors. Most nucleated cells can express various inhibitors to control complement activation on their membranes [11].

Human astrocytes and microglia from primary cultures and cell lines expressing membrane and soluble inhibitors are well protected against complement killing [11]. These cells can not activate spontaneously the complement system.

The neurons however are extremely susceptible to attack and subsequent killing by homologous complement [11]. C1q binds specifically to the membrane of neurons and leads to the activation of the complement in an antibody independent manner. Neurons and neuroblastoma cell lines

are very susceptible to complement mediated lysis showing low level of complement inhibitors [12].

In our study, we showed the presence of C1q-positive reaction in neurons of gray matter and in Purkinje cells in the cerebellum. Microglia also showed immunoreactivity localized in the granular and molecular cell layers of the cerebellum.

C3b complement factor was found mainly in neurons of gray matter, but was observed in microglia and in some astrocytes too.

Neurons presented very strong positive signal in areas of evident spongiform change. Positivity for C3b was very strong also in the granular cell layer and less evident, but still present, in the molecular cell layer of the cerebellum. Purkinje cells were often positive.

Control brains showed no complement immunoreactivity what stays in agreement with Kovacs et al. observations [8].

The evident positivity of neurons for two complement early activation factors may suggest that neuronal loss, at least in part, may be due to complement system activation.

Depletion of component C3 or genetic deficiency of C1q delays onset of scrapie [9], but this observation may be explained by the absence of complement factors on the follicular dendritic cells within germinal centers in lymphoid tissue.

Infectious agents are trapped and retained on the surface of FDCs through the interactions between complement and cellular complement receptors. Depletion or genetic deficiency of C1q and C3 delays the onset of scrapie following the peripheral infection [9].

Our findings may suggest that C1q and C3b play role also in degenerative processes within the CNS and showed the way for future investigations.

The final result of complement activation is formation of the pore in the cell membrane called membrane attack complex (C5b-9). The initial studies on our material showed also strong immunoreactivity for this factor however the following studies are mandatory.

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