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ANTIBODIES TO AXONAL NEUROFILAMENTS IN CREUTZFELDT-JAKOB DISEASE AND OTHER ORGANIC DEMENTIAS

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Summary. — Antibodies reacting with neurofilament proteins were detected by indirect immunofluorescence in the sera from 6 out of 10 patients with verified Creutzfeldt-Jakob disease (CJD), in 4 out of 8 cases of Alzheimer's disease (AD), in a variable percentage (29.4—42.8 %) of sera from patients (n=46) with other dementias of organic or infectious origin and in 5 out of 30 asymptomatic relatives of CJD patients. The occurrence of this antibody did not correlate with the duration or with any other clinical manifestation of CJD. The applicability of the test as differential-diagnostic marker appears limited. The later development of CJD and mental or nervous disease in 3 of 5 asymptomatic relatives with positive serological reaction suggest that the method although nonspecific, may be of certain value in the search for persons at higher risk to develop a degenerative disorder of CNS.

Key words: *Creutzfeldt-Jakob disease*; *antibody*; *neurofilament*; *differential-diagnosis*

Sotelo *et al.* (1980) demonstrated the presence of antibodies reacting with neurofilaments in sera of patients suffering from Creutzfeldt-Jakob disease (CJD). These antibodies, visualized in neuronal cultures by indirect immunofluorescence were the first evidence of immune response in slow virus neuroinfections caused by unconventional agents. We started to study the specificity and frequency of described antibodies in CJD replacing the cultured neurons by a standard but less laborious method, using cryostat sections of human basal ganglia (Mayer *et al.*, 1981). Present communication summarizes the data obtained from a comparative study of serum and cerebrospinal fluid (CSF) antibodies reactive with heterologous axonal antigens in slow virus neuroinfections (CJD and subacute sclerosing panencephalitis (SSPE), AD, cerebrovascular and other organic dementias as well as in asymptomatic relatives of CJD patients.

Table 1. Incidence of antibodies reactive with heterologous neurofilament protein in the sera of patients with Creutzfeldt-Jakob disease, other neurological disorders and disease-free relatives of CJD patients

Investigated disease	Number of sera		
	tested	positive*	(%)
Creutzfeldt-Jakob disease (CJD)	10	6	(60.0)
Subacute sclerosing panencephalitis	14	6	(42.8)
Alzheimer's disease	8	4	(50.0)
Cerebrovascular dementia	15	6	(37.5)
Other organic dementias	17	5	(29.4)
Retinitis pigmentosa	1	1	
Asymptomatic CJD relatives	30	5	(16.6)

* Sera were examined for reactivity with rat spinal cord by indirect immunofluorescence microscopy.

A total of 95 serum samples (from 95 persons) as well as samples of their CSF were tested. The samples were obtained from 50 clinically suspect CJD cases, 14 SSPE patients and from 30 adult relatives of patients with histologically verified CJD (without any clinical sign of neurological disease at the time of sampling). One of the relatives suffered from retinitis pigmentosa (RP). From the 50 clinically suspect CJD patients 11 have been verified as CJD, 8 as AD, 1 as Pick's disease, 15 as cerebrovascular dementia and 15 have belonged to other organic dementias.

All serum and CSF samples were screened for reactivity with heterologous neural tissue obtained from subadult Wistar rat spinal cord. Longitudinal 7 μm thick cryostat sections of spinal cord were prepared and subsequently incubated with either serum or CSF and with the fluorescein-isothiocyanate labelled $F(ab)_2$ fraction of swine anti-human IgG (Sevac, Prague). Sections mounted in glycerol were then examined in the FLUOVAL microscope (Zeiss Jena). Sera were tested in twofold dilutions starting from 1 : 4 to 1 : 32. Samples of CSF were tested undiluted and in dilutions 1 : 2 and 1 : 4. Coded slides were evaluated by the same person using grading scale: 0, +−, +, ++, +++.

The frequency of antibodies (Table 1) was found the highest (60 %) in CJD patients, considerably high (50 %) in AD and as high as 30—40 per cent in other neurological diseases. In CJD patients the fluorescence showed uniform intensity in all tested dilutions. In AD and other organic dementias the intensity of positive fluorescence staining gradually decreased with increasing dilutions of the tested sera. Positive serological reaction was observed also in the relative of CJD patient affected with RP and in 16.7 per cent of asymptomatic CJD relatives. Immunofluorescence staining pattern in CJD showed predominantly fine, sharply discernible fibrils, while in AD and in other organic dementias thicker, ribbon-like processes prevailed. No positive reaction against normal neurofilament proteins was seen in the CSF samples tested.

New nosological entities recognized as transmissible viral dementias (Gerstmann-Sträussler syndrome in humans, transmissible encephalopathy of mule deer), the significantly increased frequency of surgical interventions performed in CJD patients (Kondo and Kuroiwa, 1982; Mitrová, 1988) as well as of iatrogenic CJD in young adult recipients of human pituitary growth

hormone (Brown *et al.*, 1985), underline the need for rapid and reliable laboratory methods facilitating the differential diagnosis of infections caused by unconventional agents. Methods used at present (experimental transmission of the disease, brain biopsy and electron-microscopic demonstration of scrapie associated fibrils (SAF)), yield late results for differential diagnosis. In addition, the methods are too complicated and not available for routine laboratory practice.

A high proportion but not each of investigated CJD patients showed positive serum immunoreactivity against neurofilament proteins. No correlation of the positivity (or negativity) of antibod es with the duration or any other clinical parameter of CJD has been found. Therefore, in spite of the highest frequency of antibodies as well as certain minor differences in the staining pattern observed in CJD when compared with other organic dementias, the immune reaction with heterologous axonal antigens will not find a wider application as a specific laboratory marker of transmissible viral dementias. Comparing our findings with the data published by other authors (Bahmanyar *et al.*, 1984; Sotelo *et al.*, 1984; Toh *et al.*, 1986) there were no significant differences in corresponding groups of patients, even the methodic approaches were not identical. The positive reaction in a single patient with RP is in accordance with observations described previously (Galbraith *et al.*, 1986; Mitrová, 1988).

Gajdusek (1986) assumed that if the disturbance of axonal flow underlies neurofibrillary tangle formation and even motor neuron degeneration, the autoantibody reactivity may be the result of a pre-existing B-cell activation by release of the neurofilament components caused by interference with their transport down the axon by any of the agents. This assumption seems to provide a plausible explanation for the presence of antibodies against neurofilaments in sera of 5 disease-free relatives of CJD patients. In the course of two years one of them developed CJD and other two schizophrenia or a severe neurasthenic syndrome. The last two have shown no clinical signs of neurological or mental disorder as yet.

Considering this observation, the studied serum reactivity, which appears to be without a significant differential-diagnostic value, may prove useful from a predictive aspect in persons at higher risk to develop nervous or mental disease. Such a group is represented e.g. by family members of CJD patients, especially from the temporo-spatial accumulation of the disease, where the percentage of the familial form is as high as 35 per cent (Mitrová *et al.*, 1986). Observations of the appearance of anti-neurofilamentous antibodies during asymptomatic phase of experimentally induced subacute spongiform encephalopathies (Aoki *et al.*, 1982) are consistent with our assumption.

We conclude that determination of serum reactivity with neurofilament proteins, despite of its low importance as a specific diagnostic marker for infections caused by unconventional agents, may contribute to a better understanding and perhaps also to an earlier recognition of initial manifestation of degenerative processes of neurofibrils, developing in slow virus

infections. Further investigations are needed in order to prove its informative and preventive value in persons at professional or familial risk of CJD.

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