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AUTOANTIBODIES AGAINST NEUROFILAMENTS IN CREUTZFELDT-JAKOB DISEASE: IMMUNOFLUORESCENCE IN SECTIONS OF HUMAN CAUDATE NUCLEUS

[1,3]V. MAYER, [1]J. RAJČÁNI, [2]E. MITROVÁ

[1]Institute of Virology, Slovak Academy of Sciences, 809 39 Bratislava; [2]Research Institute of Preventive Medicine, Bratislava; and [3]Joint Virus Research Centre at the Clinic of Infectious and Parasitic Diseases, Medical Faculty, Komenský University, Bratislava, Czechoslovakia

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Summary. — Heterospecific autoantibodies to axonal neurofilaments of neurons in sera from five patients with Creutzfeldt-Jakob disease were detected with the use of cryostat section from normal human brain caudate nucleus. The antibody, visualized by the indirect immunofluorescence technique, was shown to bind complement. This allowed to achieve brighter staining either by the anticomplementary method alone or by a combination of both anti-C3 and anti-IgG conjugates.

Key words: Creutzfeldt-Jakob disease; neurofilament protein; autoantibody; immunofluorescence

Introduction

The pathogenesis of the neurohistological and clinical manifestations of the Creutzfeldt-Jakob disease (CJD) has not yet been clearly defined. Due to the unusual properties of the agent(s) of transmissible spongiform encephalopathies (TSE) and to a failure to detect a specific immune response in the afflicted host, these lethal nervous disorders have been considered as immunologically mute. Specific autoantibodies (AA) against the axonal neurofilament protein, however, were detected in sera from a considerable number of patients with CJD and kuru as compared with fewer sera from patients with certain, mainly degenerative disorders of the central nervous system (CNS) as well as in sera from some healthy persons (Gajdusek, personal communication, 1979; Sotelo *et al.*, 1980). Human AA were found heterospecific as they reacted with the neurofilaments in neurons of mice, rats and hamsters cultured in vitro. These findings were extended by Bahmanyar *et al.* (Gajdusek, personal communication, 1981), who detected AA in sera from patients with similar diseases, using sections of the rat spinal cord.

Our studies were aimed at confirming these findings with sera from CJD patients in Slovakia and other patients with miscellaneous neurological conditions. Sera were applied to sections of fresh human basal ganglia, in which

neurons of the caudate nucleus served as substrate for detection of AA by the indirect immunofluorescence (IF) and anti-complementary immunofluorescence (ACIF) methods. The use of a conjugate to guinea pig C3 component of the complement yielded brighter staining of the neurofilaments.

Materials and Methods

The sera investigated were obtained from 5 patients (3 males, 2 females, designated CJD-1 — CJD-5), with clinically typical CJD, confirmed at autopsy. Two of them, CJD-2 and CJD-3, were cases of sporadic CJD, the remaining three originated from an area of higher incidence of the disease (Mayer *et al.*, 1979; Mitrová, 1980). Blood was drawn from the stuporous patients several days before their death. The ailment, with an ingravescent course, lasted on the average 3.9 months (range 1.5—6 months) and the mean age of CJD patients at the time of death was 54.2 years. Further sera were obtained from spouses and children of the CJD patients. For comparison, 18 human sera were examined; 11 were obtained from patients with a variety of neurological disorders (kindly supplied from in-patient neurological services in Bratislava and Piešťany); 2 sera were from patients with systemic lupus erythematosus showing CNS dysfunctions (kindly supplied by Dr. J. Rovenský, Research Institute of Rheumatic Diseases, Pieš°any), 2 sera were from normal subject and 3 from patients with various non-neurological medical conditions. All sera were heated 56 °C for 30 min and stored in portions at —18 °C until tested.

In the indirect IF and ACIF methods we used sections of human brain caudate nucleus; mouse and rat basal ganglia; and mouse liver and kidneys. Normal human brain tissue was obtained at autopsy of subjects not suffering from CNS disease, provided that the bodies were kept in a refrigerator for not more than 4—6 hr. Rectangular slices (5 × 10 mm), prepared from the caudate area and thoroughly washed, were immediately quickly frozen in liquid propan-butan and stored on dry ice. Six μm thick sections were cut in a cryostat, fixed in acetone for 10 min at 4 °C and further processed either immediately or after overnight storage at —18 °C. Before staining, all sera were absorbed with calf liver powder. Sections were treated with serum, usually diluted 1 : 5 (in titrations 1 : 10, 1 : 20 and 1 : 40), for 30 min and then washed three times in phosphate-buffered saline (PBS), pH 7.2. The first series of sections was stained with a conjugate to human Ig (SwAHU, SEVAC, Prague). The second series was treated with commercial guinea pig complement (diluted in saline to achieve 2 units per 0.1 ml) for 40 min at 37 °C, washed three times and treated with a conjugate to the third component (C3) of guinea pig complement (Dynatech, Switzerland), diluted 1 : 10 in saline. The third series of sections, after treatment with anti-C3 conjugate, was washed in PBS and, finally incubated with a conjugate to human IgG (SwAHu-IgG, SEVAC, Prague). Sections stained with (a) only either conjugate; (b) somplement and either conjugate; and (c) complement and both conjugates were included as controls. Furthermore, in repeated staining trials we tested selected sera absorbed to group A streptococci to remove cross-reactive antibodies to neurons, which may mimic AA activity (Kingston and Glynn, 1976; Husby *et al.*, 1977). Sera were checked for antinuclear antibodies using mouse cerebellar cortex tissue and the two lupus erythematosus sera as positive controls. The interspecies specificity was tested on sections from basal ganglia of mice and rats, and any possible heterologous nonspecific interactions were checked on liver and kidney sections. Results were read either immediately of after overnight storage at 4 °C.

Results and Discussion

Binding of AA to axonal neurofilaments and to perinuclear cytoplasm of neurons of the normal human caudate nucleus was observed in all sera from CJD patients (Figs 1 and 3). The brightness of the fluorescence varied (Table 1). The titres of AA in the sera from patients CJD-4 and CJD-2 did not exceeded 10 or 20 respectively. The absorption to group A streptococci did not influence the results. The fluorescence in sections of mouse and rat

Table 1. AA to axonal neurofilaments of normal human caudate nucleus neurons in sera of CJD patients

Patients, age in years	Duration of dementia-myoclonic phase (months)	AA*	Findings
CJD-1, 58	5–6	++	Bright fibrillar fluorescence in axons and granular fluorescence in the cytoplasm of neurons (Figs 1, 3).
CJD-2, 54	6	+	Cytoplasmic perinuclear fluorescence in neurons and fine axonal fluorescence (Fig. 2).
CJD-3, 62	4	+	Fibrillar fluorescence in neuronal processes and perinuclear fluorescence in the cytoplasm.
CJD-4, 49	1.5	(+)	Frequent fibrillar fluorescence in axons and neuronal processes.
CJD-5, 48	2.5	+	Fluorescence in axons and in the perikaryon of neurons.

* AA detected by indirect IF; (+), + and ++: fluorescence intensity increasing in that order.

basal ganglia was less bright but unequivocally positive. This is consistent with the findings by Sotelo *et al.* (1980) and Bahmanyar *et al.* (Gajdusek, pers. comm., 1981), who described a similar heterospecificity of AA. No nuclear staining was seen with CJD sera, but staining of the small vessel walls (reticulin?) in the brain and in kidney glomeruli was relatively frequent in sections stained by the ACIF method. All CJD sera, as well as some control sera, including those from healthy persons, stained the glial processes (astrologia, Fig. 4). Obviously, such heterologous reactions are the disadvantage of section staining as compared to the staining of isolated neurons in culture (Sotelo *et al.*, 1980). The latter technique is more exacting and appears less versatile for routine use. In further investigations it is advisible adsorb the test sera with glial fibrillary acidic protein to diminish non-specific glial staining on the substrate used. The reaction with the vessel walls could be abrogated by absorption to renal cortex suspension and by counterstaining with thiazine red.

Sera from unaffected family members, namely one husband, two wives and four adult children from families with a CJD case were also investigated for AA:

CJD-1:	son, 38 years old	AA absent
CJD-2:	husband, 57 years old	AA absent
	daughter, 21 years old	AA absent
	daughter, 26 years old	AA present
	son, 35 years old	AA present
CJD-4:	wife, 45 years old	AA absent
CJD*:	wife, 67 years old	AA absent

*Serum from this 62 years old patient was not available. The dementia-myoclonic phase lasted for about three months before his death; CJD was confirmed at autopsy.

Of these seven sera, only two (a daughter and a son of the CJD-2 patient) were positive for AA. It appears of interest that this son suffered for years

from severe neurasthenia. Obviously, it is too early to evaluate the eventual clinical value of such findings, because AA were found in 8—10% of investigated sera from apparently healthy persons (Sotelo *et al.*, 1980; Bahmanyar *et al.* — Gajdusek, pers. comm., 1981). Nevertheless, the data obtained so far warrant further extensive investigations. This seems to be of particular interest in view of the established stigmatization of the neuropsychic sphere in some members of families with sporadic and familial CJD cases (Mitrová, 1980), the genetically determined susceptibility to the disease (Gajdusek, 1977; Brown, 1980) and even the unusually long preclinical period with replication of the causative agent in the CNS. Sotelo *et al.* (1980) and Bahmanyar *et al.* (pers. comm. 1981) examined large collections of sera and detected AA in 45—55% of sera from CJD patients, in 24—54% of those with kuru, but only in 13—27% of cases of other, prevalently degenerative CNS disorders. In Huntington's disease, the presence of antineuronal antibodies depended clearly om the progression of the disease (Husby *et al.*, 1976) and it is possible that also other variables may influence the appearance of AA. We examined sera from 11 patients with miscellaneous neurological diseases (amyotrophic lateral sclerosis, multiinfarct dementia, olivo-ponto-cerebellar degeneration, brain reticulosarcoma, multiple, sclerosis, myelitis, CNC polytraumatism, spastic quadruplegia, syringomyelia, sequelae after meningitis basilaris) and found AA in one case (9%) of Huntington's disease.

According to the evidence available, generation of AA reacting with the neurofilament protein of normal central neurons appears as a nonspecific phenomenon, emerging more frequently during the progressive course of organic nervous disease bearing a degenerative character, but particularly accentuated in the CJD situation. This suggests that an immune dysfunction may play a role in this disease or in its aggravation. It remains obscure whether circulating AA are an event secondary to the damage of the blood-brain barrier systems and related to virus-induced destruction of axons. Furthermore, the reason for the presence of AA in sera from some normal persons is unknown. A simple relatedness af AA to the age-dependent brain tissue autoantibodies, observed in certain proportion of elderly persons, seems improbable, taking into account the findings in matched controls. AA in TSE, however, may reflect the early appearing, greatly accelerated and much more extensive development of qualitative changes in nervous tissue, which are associated — but considerably less expressed — physiologically with the advanced aging of the brain. Systematic studies are necessary to evaluate the participation of AA in the pathogenesis of slow viral diseases of the CNC, caused by both conventional and unconventional agents and their eventual diagnostic significance.

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Explanation of Photomicrographs (Plate LVI):

Fig. 1. Fluorescence in the cytoplasm of a neuron and in the axonal processes of another two neurones. Serum CJD-1, ×120.

Fig. 2. Fluorescence in the body of neurons and in the neurite of a pyramidal cell. Serum CJD-2, ×120.

Fig. 3. Fluorescence in two neurons and in the neurite of one of them (arrows). Serum CJD-1, ×120.

Fig. 4. Fluorescence in the cytoplasm of a neuron and in the processes of an astroglial cell. Serum CJD-2, ×120.