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DEVELOPMENT OF ANTIBODIES TO AXONAL NEUROFILAMENTS IN THE PROGRESSION OF CHRONIC TICK-BORNE ENCEPHALITIS

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Summary. – We followed the presence of autoantibodies to neurofilaments (NF) in the sera of patients with acute tick-borne encephalitis (TBE), chronic TBE, amyotrophic lateral sclerosis (ALS), and other diseases of CNS. The diagnosis was made according to clinical signs and based on virusneutralizing antibodies. Autoantibodies to NF were found in the majority of chronic TBE patients during disease progression, but were neither present in acute TBE nor in chronic TBE cases during the stabilization phase. Autoantibodies to NF found in a patient with acute TBE showed subsequent progression to a prolonged course. The data are discussed in order to assess the mechanisms of the chronic TBE process and its role in impairing the slow axonal transport.

Key words: acute and chronic tick-borne encephalitis; autoantibodies to neurofilaments; pathogenesis

Introduction

It is known that tick-borne encephalitis (TBE) may take an acute or a chronic course. The frequency of chronic progredient forms of TBE varies in different regions of the U. S. S. R. from 1–14 % (Umansky and Dekonenko, 1983). The main interest of investigators following the development of chronic tick-borne encephalitis was devoted to the role of virus persistence and its mechanisms. Less attention was devoted to the role of macroorganism in this process, although it seems reasonable to assume that chronic TBE is associated with genetically determined failure of the immune system to eliminate the virus (Umansky and Dekonenko, 1983). Autoantibodies to neurofilamentary proteins, which are important structural and functional components of neurons, were described in several chronic and slow CNS infections (Bahmanyar *et al.*, 1984; Mayer *et al.*, 1981; Sotelo *et al.*, 1980; Aoki *et al.*, 1982; Roikhel *et al.*, 1985; Votiakov *et al.*, 1987; Plioplys, 1987; Anderson and Posner, 1988).

However, such autoantibodies were not yet described in flavivirus infections. It can be expected that lesions in the cytoskeleton of neurons may occur in initially progredient forms of TBE as well as after overcoming the acute phase of infection which, in turn, may be followed by the occurrence of autoantibodies to neurofilaments. Here we report on the occurrence of autoantibodies in the serum of patients with chronic TBE. As controls we examined the sera of patients with AML and other diseases of CNS.

Materials and Methods

Sera were coming from 21 patients hospitalized in the Department of Neurology of Sverdlovsk First Regional clinic. Blood was drawn during the acute phase of encephalitis within the first weeks of disease and later on by 3–4 months since onset, i. e. when residual signs of disease were present in the absence of any signs of progression. In patients with chronic course of TBE blood was drawn during hospitalization. The diagnosis of TBE was based on clinical and laboratory examinations. The sera were kept at −20°C.

Serologic reactions. Neutralizing antibodies were detected by 2 methods, i. e. by testing in mice and on microplates. Mice were given intracerebrally the mixtures of TBE virus and serum (starting from dilution 1:4 at different virus dilutions) after incubation for 1.5 hr. The virusneutralization index was determined in log LD_{50} units per 0.03 ml. Neutralization reaction in SPEV cells was performed on 96-well microplates (Costar). Twofold serum dilutions were mixed with 100 $TCID_{50}$ of TBE virus incubated for 1.5 hr at 37°C and inoculated into the cells grown in Earle's solution. The neutralizing antibody titre was expressed in units per well suppressing CPE. The Sofjin strain was used as indicator virus.

Autoantibodies to neurofilaments were detected according to Sotelo *et al.* (1980) in the modification of Mayer *et al.* (1981). We used cryostat sections from the mouse spinal cord; anti-human IgG, FITC-labelled IgG fraction (Gamalea Institute of Epidemiology and Microbiology, Moscow) was used as conjugate. The sections were contrasted with rhodamine-labelled calf albumin.

Results

Table 1 shows the clinical classification of the patients in question. The clinical diagnosis of chronic TBE was made in 9 patients based on a several months lasting course of the disease since tick bite and a slowly developing symptomatology. In 6 cases with sudden onset of the disease, TBE was classified as acute; in 4 cases diagnosis AML was based on clinical investigations and in 2 cases the aetiology of neuroinfection has remained uncertain. The diagnosis of TBE was confirmed by serological findings. The neutralization index to TBE virus in patiens with AML ranged from 1.67 to 3.17 log units. This antibody level to TBE virus and that found in the other "control" group was in the same range as the neutralization indices determined in large surveys of healthy population in a focus of the Sverdlovsk region (Pogodina, 1966).

Autoantibodies to NF were detected in the sera of 6 out 15 patients with acute and chronic TBE. In the group of 9 chronic TBE patients, 5 out 6 had autoantibodies to NF in the phase of progression (at the time of serum taking),

Table 1. Frequency of autoantibodies to NF and the level of specific humoral immune response in patients with TBE and other CNS diseases

Disease	No. of patients With autoantibodies to NF per total No. of patients	Mean neutralization index to TBE virus log LD_{50}	Mean titre of virusneutralizing antibodies units/well
Chronic tick-borne encephalitis			
- progression phase	5/6	4.28	173
- stabilization phase	0/3	4.68	256
Acute TBE	1*/6	4.38	224
Amyotropic lateral sclerosis	3/4	2.54	85
Other diseases of CNS	1/2	2.75	32

* in this patient within 3 months since the acute disease progression of infection occurred continuing into chronic TBE.

but none had autoantibodies in the of stage stabilization, although in both cases high levels of neutralizing antibodies were found. Only 1 out 6 patients with symptoms of acute TBE revealed autoantibodies to NF. This particular patient (P) in the acute period showed tetraplegia, coma, and convulsive twitches. Within 1 month since partial recovery and improvement of extremity movements, signs such as nystagmus, weakness of cervical muscles, high tendon reflexes, and psychical impairment continued throughout. Within 2 months cerebellar symptoms appeared accompanied with myoclonal hyperkinesis of one extremity, general convulsive paroxism and loss of consciousness. Finally, within 4 months the Kozhevnikov's epilepsy syndroma developed confirming chronic progression of disease. It should be noted that autoantibodies were found in the serum on day 25 since the onset of disease, i. e. before the chronic outcome; therefore, we included this result into the group of acute cases.

Autoantibodies to NF were also found in 3 out 4 patients with ALS, which was consistent with our earlier findings (Roikhel *et al.*, 1985; Zavalishin *et al.*, 1988). Autoantibodies to NF were detected also in the serum of a patient with neuroinfection of unknown aetiology which showed the signs of Landry syndrome.

We tried to analyse the possible influence of the infection phase on the frequency of autoantibodies to NF. Therefore, all patients were grouped according to the progression or regression of their disease. Autoantibodies occurred

in 10 out of 12 patients with progression of their disease but in none out of 9 patients with stabilized nonprogressing course.

Discussion

Different explanations are available in the literature for understanding of the mechanisms of chronic forms of TBE from admitting the essential role of the virus to assuming its triggering effect. These viewpoints on the pathogenesis of chronic TBE have been summarized by Pogodina *et al.* (1986). A possible aspect for following the pathogenesis of chronic TBE might be the investigation of functional activity of neuronal cytoskeleton in the area of the pathological process. One of the indicators of functional state and integrity of subcellular components of neurons is the appearance of autoantibodies to NF. The analysis of our experimental data has shown that during the acute phase no autoantibodies develop regardless to the destruction of neurons. Contemporanily in chronic TBE patients autoantibodies appeared in a considerable amount during the progression of disease. In our patient P. (Table 1) autoantibodies to NF appeared already in the early phase of his disease, although at this stage no signs of progression to chronic disease were detectable. Despite of that this patient developed chronic TBE. This supports our impression on the prognostic value of the detection of autoantibodies to NF in TBE.

As shown by Pogodina (personal communication) the serum of monkeys with chronic TBE more frequently contained antibodies to normal brain detected by complement fixation reaction as did the normal monkey serum. Autoantibodies against the transiently occurring complement binding brain antigens were detected in the sera from patients with progredient TBE in their phase of progression (Artemikin and Baishtuk, 1972).

It may be assumed that changes of NF belong to the accompanying events of chronic TBE. The NF about 10 nm thick are composed of 3 proteins with M_r 200, 150, and 70 kD and associate with an additional protein of M_r 62 kD. NF are involved in the slow axonal transport of lysozomes, enzymes, and neurotransmitters to the axon terminals. Interference with the axonal transport is one of the basic mechanisms acting in slow virus infections and other degenerative diseases of CNS. As interfering factors may come into account either classical viruses or prion proteins; one sign of the impaired axonal transport is the release of NF proteins and corresponding autoantibody formation (Gajdusek, 1984; 1985; Toh *et al.,* 1985; Liberski *et al.,* 1987).

When considering the persistence of RNA-containing viruses in the CNS autoantibodies to NF were noted in certain infections (Chan, 1985; Kristensson and Norrby, 1986). Alterations in axonal transport under the influence of viral infections in particular have been described in Semliki forest virus infection (Jenkins *et al.,* 1988). When examining the neural spread of Powassan virus from the TBE complex its distribution was detected in

neurons, along the axons, and in the dendrites (Sobolov and Shestopalova, 1978). It can be assumed that the virus acts on the neurofilaments during its activation in the course of transition to subacute or chronic infection. This can be confirmed by our finding of autoantibodies to NF during the progression of CNS infection in chronic TBE. Possibly, the involevement of NF may be reversed because the antibodies to NF do not develop at stabilization of infection process.

Based on these data we may assume that the development of chronic TBE process may show some common features to slow and degenerative neuroinfections of CNS as manifested in the alteration of slow axonal transport; an indirect indicator of such feature is the occurrence of autoantibodies to structural axon components.

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