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FIXATION OF HUMAN SERUM ANTIBRAIN ANTIBODIES IN DIFFERENT PARTS OF THE RABBIT BRAIN

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The indirect immunofluorescence method of Coons was used to study the character of fixation of antibrain antibodies (ABA), contained in the sera of patients with essential hypertension, amyotrophic lateral sclerosis, multiple sclerosis, hepatocerebral degeneration, and myoclonia epileptica on rabbit brain sections. ABA complementary to different structures of nerve tissue were always formed in these diseases. The antigenic properties of the individual brain tissue components of the patients were not altered to the extent of differing completely from the entigenic properties of the same formations in normal animals. Meanwhile different components of nerve and glial cells, the myelin sheaths of various conducting systems and, to a lesser degree, cells of the ependyma and walls of blood vessels located in different parts of the brain possessed not only common, but also different antigenic propeties.

KEY WORDS: antibrain antibodies; essential hypertension; amyotrophic lateral sclerosis; multiple sclerosis; hepatocerebral degeneration; myoclonia epileptica.

More than 10 water-soluble and water-insoluble brain-specific antigens have now been found and identified in the tissues of the central and peripheral nervous systems: proteins S-100 and 14-3-2, BE-antigen, α_2-glycoprotein, α-antigen, and so on [3,6,9,12,13,17-19]. Not only antigens of gray and white matter, but also antigens of various parts of normal and pathologically changed brain tissue have been determined by various methods [1,5,7,14-16]. Meanwhile the location of the antigenic determinants in nerve and glial cells, in myelin sheaths, and in the walls of blood vessels situated in different anatomical formations of the brain has still received little study [4,8,11,20].

The object of this investigation was to determine the fixation sites of antibrain antibodies (ABA) on the above-mentioned tissue structures of the brain in order to ascertain their antigenic similarity and differences.

EXPERIMENTAL METHOD

Coons' immunofluorescence method in its indirect modification was used [10]. Whole blood sera from 10 patients with essential hypertension, 16 with amyotrophic lateral sclerosis (ALS), 12 with multiple sclerosis (MS), seven patients with hepatocerebral degeneration (HCD), and four patients with myoclonia epileptica were used as the AMA. Blood sera from 50 donors, the results of testing which have already been reported [4], were used as the control.

Frontal sections of unfixed rabbit brain taken 1-2 min after decapitation were used as the nerve tissue antigen. After rapid cooling of the brain on dry ice, sections were cut in a cryostat at −20°C. Sections for investigation were taken at the level of maximal development of cortical area 4, at the level of the caudal part

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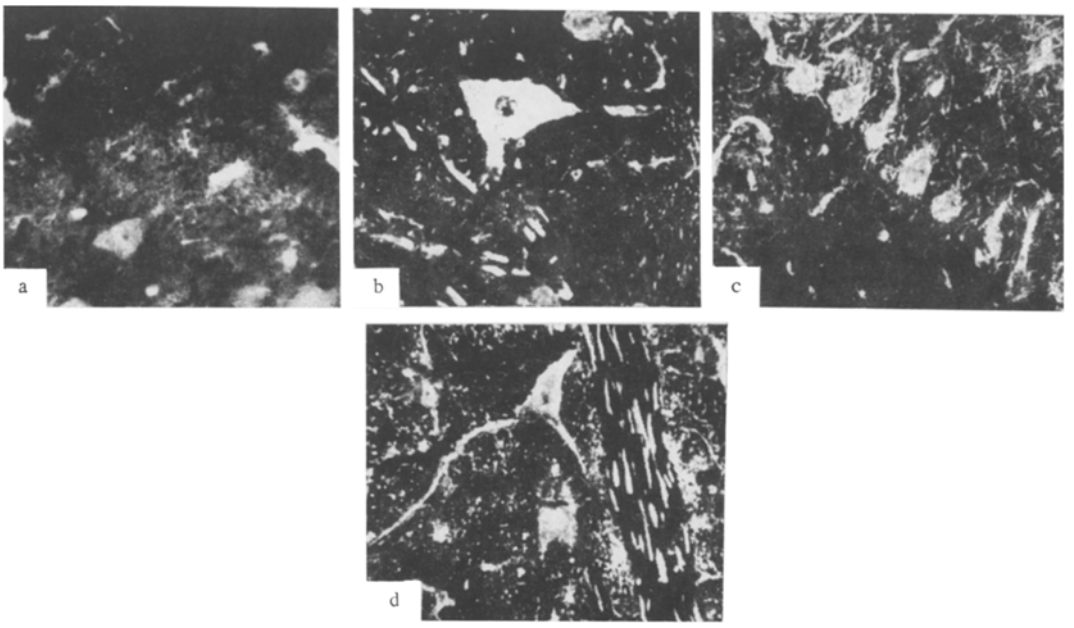

Fig. 1. Character of fixation of ABA from a patient with MS on neurons in different parts of rabbit brain: a) very weak fluorescence of neurons in cortical area 4; b) very bright fluorescence of motoneuron from nucleus of abducens nerve; c) less bring fluorescence of Purkinje neurons; d) bring fluorescence of neuron in n. reticularis pontis caudalis with equally bring fluorescence of blood vessels, increased intensity of fluorescence of myelin sheaths, and certain differences in fluorescence of glia. Indirect Coons' method, magnification: objective 20 ×, ocular homal 3 ×.

of the pons, i.e., in accordance with plates 28 and 12 of the "Atlas of the rabbit brain" compiled by Blinkov et al. [2], and also at the level C4-C5 of the spinal cord.

The serum was incubated with the brain sections in a moist chamber at 37°C for 40 min. After careful washing in physiological saline (pH 7.2-7.4) to remove serum, the sections were stained to reveal the antigen–antibody complex with a commercial antiserum against human globulins labeled with fluorescein isothiocyanate (N. F. Gamaleya Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR) at 37°C for 40 min. The sections were also carefully rinsed to remove the luminescent serum, after which they were mounted in 50% glycerol solution with buffered physiological saline, examined, and photographed in the ML-2 luminescence microscope.

The controls usually used with the indirect Coons' method were carried out: 1) staining the complexes formed on the section heterologous luminescent serum; 2) destruction of the immune complexes by incubating the sections before staining in an acid buffer solution (pH 3.5); 3) exhaustion of the sera with brain tissue homogenate in order to adsorb ABA from them before incubation with the brain sections. Fluorescence was regarded as specific if it was extinguished in all the control tests. The intensity of luminescence was estimated by menas of a 4-point system.

EXPERIMENTAL RESULTS

It was reported previously [4] that the ABA of blood sera from the 50 donors had no affinity for all brain tissue components simultaneously in any single case. Moreover, in 17 of the 50 cases they contained no ABA whatsoever, in four cases they had affinity only for myelin, in nine only for blood vessels, in 16 for myelin and blood vessels, and only in four cases affinity for neurons, myelin, and blood vessels. By contrast, ABA of the blood serum of the neurological patients had affinity in all cases for neurons and glial cells, myelin, and blood vessels; in the different nosological forms this affinity differed and, in addition, differences often were found in the character of fluorescence of the same elements along the length of the brain.

The ABA from patients with essential hypertension (lasting 10 years or more, with a high blood pressure), for instance, were fixed equally to the cytoplasm of neurons in all parts of the brain studied and gave weak fluorescence (±, +). ABA of patients with ALS were fixed on the cytoplasm and some intracellular and intra-

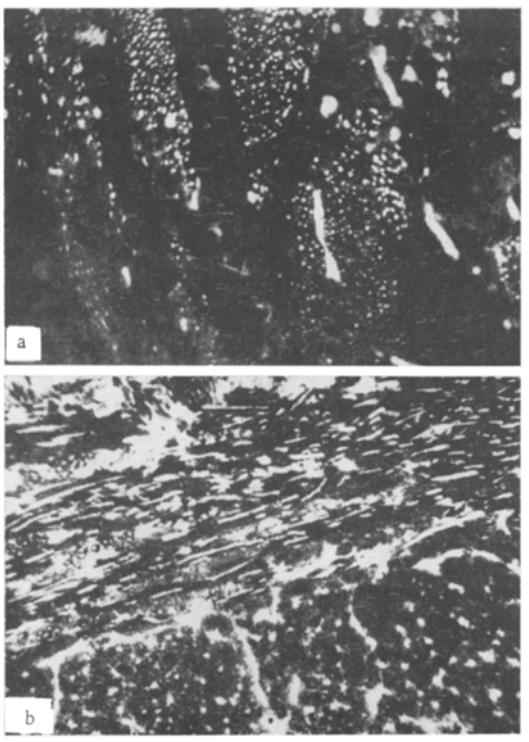

Fig. 2

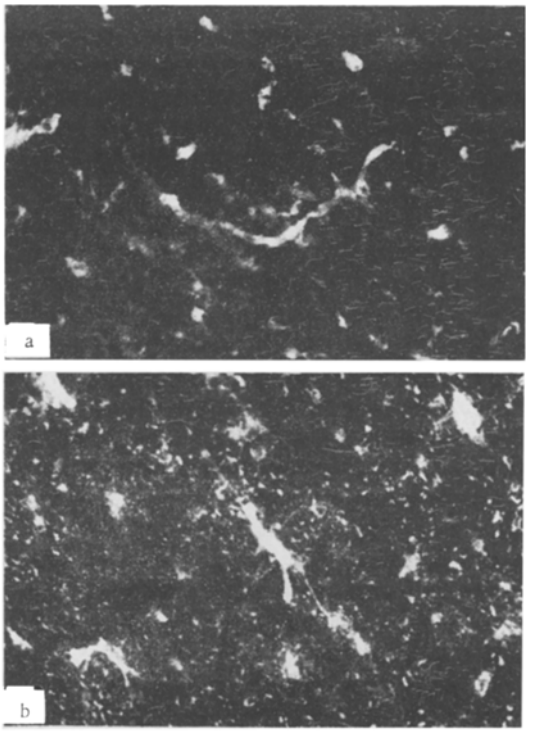

Fig. 3

Fig. 2. Fixation of ABA on myelin sheaths in various fiber systems accompanied by bright fluorescence of blood vessels: a) very bright fluorescence of some myelinated fibers with absence of fluorescence of others lying alongside in centrum semiovale of rabbit brain (section incubated with serum from patient with ALS); b) very bright fluorescence of myelinated fibers of pontine nuclei accompanied by weak fluorescence of myelinated fibers of pyramidal tract (section incubated with serum from patient with MS). Indirect Coons' method, objective 20 ×, ocular, homal 3 ×.

Fig. 3. Character of fixation of ABA of patient with HCD of astrocytes of different parts of rabbit brain: a) absence of fluorescence of astrocytes in cortical area 4; b) bright fluorescence of astrocytes in underlying white matter, accompanied by bright and uniform fluorescence of blood vessels. Indirect Coons' method, objective 40×, ocular homal 3×.

nuclear structures, as a result of which the shape of the neurons was clearly visible. In each case motoneurons of the cranial nerves had brighter fluorescence (2+, 4+) than the cells of the sensory nuclei and brain-stem reticular formation located alongside them (+, 2+). Neurons of the cerebral cortex, caudate nucleus, and cerebellum gave weak fluorescence (+, 2+) during investigation of only five of the 16 sera. Sera of all patients with MS likewise had affinity for neurons, which had the typical appearance of neurons stained by the Nissl method. The intensity of fluorescence of the neurons along the length of the brain was almost the same when sera from patients with the cerebral form of MS were used and it rose sharply in a caudal direction in the spinal form of MS. Meanwhile, in every case the motoneurons gave brighter fluorescence (3+, 4+) than cells of the sensory nuclei and nuclei of the reticular formation (2+, 3+; Fig. 1). ABA of sera of patients with HCD also had affinity in all cases for neurons, their cytoplasm was homogenous in appearance, and their Nissl bodies did not give fluorescence. The membranes of the nucleus and nucleolus usually were fluorescent. The intensity of fluorescence rose in the caudal direction although when sera from patients with cortical lesions were studied there was almost identical affinity of the ABA for both cortical neurons and cells of the rabbit brain stem. The ABA of sera from patients with myoclonia epileptica had affinity for Nissl bodies and the nuclear membrane only of neurons in the brain stem. The intensity of fluorescence was weak (+, 2+). In other parts of the brain no fluorescence at all was found.

ABA complementary with the myelin sheaths were found in the sera of all patients studied. The intensity of fluorescence differed in different cases. The axons gave no fluorescence and for that reason myelinated

fibers appeared like hollow tubes, cut across at different angles. Thick myelinated fibers constantly had brighter fluorescence than thin fibers. Fluorescence of myelin in the centrum semiovale and in the pyramidal tract most frequently had much weaker fluorescence than the other fibers of the brain stem: the trapezoid body, the medical and lateral lemnisci, the roots of the cranial nerves. In essential hypertension antimyelin AMA were found only in patients ill for a long time. A special feature of the antimyelin AMA in ALS was their affinity for some fibers but absence of affinity for others lying side by side and performing the same or different functions. In MS no such differences in the fluorescence of the sheaths of the fibers could be seen. In HCD equally bright fluorescence was found over the whole brain. In myoclonia epileptica fluorescence of myelin was found only in fibers in the brain stem and its intensity was weak (Fig. 2).

Fluorescence of glial cells differed in the patients with all the various diseases. In essential hypertension only very weak fluorescence of the nuclear membrances of the glial cells and the adjacent rim of cytoplasm was found throughout the brain, so that the type of glia could not be identified. In ALS the ABA in all cases had affinity for the fibrous astrocytes of the white matter. In areas of gray matter lying next to them no astrocytes were found. The intensity of fluorescence was very strong only in one case (4+). In all cases of MS bright fluorescence of the fibrous astrocytes, with all their characteristic processes, was found in the white matter (3+, 4+). In the gray matter the astrocytes gave fluorescence when tested with only three sera. Bright fluorescence (2+, 3+) of the astrocytes mainly in the region of the basal ganglia was observed when the sera of all patients with HCD was studied. In myoclonia epileptica fluorescence of the glial cells was found only among fibers of the brain stem. It was very weak (±, +) and did not reveal the characteristic features of any particular type of glia (Fig. 3).

Bright fluorescence of the ependymal cells and of the subependymal fibrous structure was found during the study of sera from patients with ALS and MS.

Affinity of the ABA for the walls of the intracerebral blood vessels was revealed in all cases. The blood vessels had the appearance of hollow tubes with a sharp shadow at the site of the endothelial nuclei. In essential hypertension, ALS, HCD, and myoclonia epileptica fairly uniform fluorescence of the blood vessels was found in all parts of the brain but it was always brighter in the white matter. In MS there was a more or less marked increase in fluorescence of the blood vessels in the caudal direction.

In organic diseases of the human nervous system an immune response with the formation of ABA complementary to all tissue structures of the rabbit brain is thus always present. The pathogenetic role of this immune response is not clear, but it is evidently not identical in the different nosological forms. The character of fixation of the ABA in these nosological forms in fact differs depending on their affinity for nerve and glial cells, myelinated fibers, and blood vessel walls, and not only by their biological action on the brain tissue of animals when patients' sera are injected intracerebrally into them [4]. It also differs from the results observed by the present writer [4] and others [8,11,20] during incubation of donors' sera.

Although ABA by means of which the antigenicity of the various brain formations could be judged were serum antibodies synthesized in patients with various lesions of the nervous system, they were fixed on sections of normal rabbit brain and could be removed from the sera by their exhaustion with normal brain homogenate. In other words, the antigenic properties of individual structures of brain tissue in diseases of the nervous system are not altered sufficiently so that they differ completely from the antigenic properties of the same formations under normal conditions. Meanwhile the investigation showed that different components of different nervous and glial cells, the myelin sheaths of different fiber systems and, to a lesser degree, the cells of the ependyma and blood vessels, located in different parts of the brain, have not only common but also different antigenic properties.

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