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EFFECT OF ANTIBRAIN ANTIBODIES ON LIPID PEROXIDATION IN THE BRAIN

G. A. Vilkov, E. M. Stepanenko, and T. A. Khoruzhaya

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KEY WORDS: antibrain antibodies; blood serum; brain; lipid peroxidation.

Disturbance of the functional and structural integrity of the neuroglial cell membrane in multiple sclerosis and experimental allergic encephalomyelitis (EAE) has been demonstrated in many investigations [2, 4]. There are also insolated publications reporting intensification of lipid peroxidation (LPO) in patients with multiple sclerosis and schizophrenia, and this may be a factor involved in modification of the membranes and various disturbances of brain functions [5, 6].

The blood sera of patients with multiple sclerosis and schizophrenia, and also of animals with EAE are known to have an injurious membranotropic action which, according to some authorities, is due to a factor (factors) contained in the γ-globulin fraction of serum [7, 9, 10]. One such factor may be the antibrain antibodies that are found in the serum of patients with schizophrenia and multiple sclerosis [3, 4].

The effect of blood sera of normal dogs and of dogs immunized with brain, of IgG isolated from these sera, and of blood sera from patients with neuropsychic diseases, with certain somatic diseases, and of normal subjects, on LPO in brain tissue *in vitro*, and also in effect of the IgG fraction isolated from blood serum of dogs immunized with brain tissue on LPO processes after intracisternal injection, were studied in the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-180 g. The relative antioxidant properties of the blood sera were judged by their ability to inhibit LPO in homogenates of surviving rat brain and liver tissue. The incubation medium contained 0.5 ml of a 3.3% homogenate made up in 0.05 M Tris-HCl buffer, pH 7.4, 0.3 ml of the test blood serum,

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TABLE 1. RAA of Blood Serum (relative units) of Normal Subjects and Patients with Various Somatic and Neurophysic Diseases (M ± m)

Diagnosis	Incubation with brain homogenate	Incubation with liver homogenate
Control	0,59±0,01	0,36±0,01
Schizophrenia	0,33±0,02*	0,34±0,01
Boundary states (neuroses, reactive states) (10)	0,48±0,01	0,36±0,03
Multiple sclerosis	0,39±0,03*	0,35±0,02
Benign brain tumors (10)	0,51±0,01	0,35±0,01
Syringomyelia (3)	0,54±0,02	—
Osteochondriosis (7)	0,59±0,03	—
Myocardial infarction (7)	0,52±0,02	0,36±0,01
Psoriasis (6)	0,59±0,02	0,35±0,01
Infectious hepatitis (10)	0,57±0,01	0,21±0,02*

Number of patients studied given in parentheses. Here and in Tables 2 and 3: *P < 0.001 compared with control.

TABLE 2. RAA of Blood Serum (relative units) of Patients with Schizophrenia and Multiple Sclerosis after Incubation with Homogenates of Gray and White Matter of Rat Brain (M ± m)

Diagnosis	Gray matter	White matter
Control	0,52±0,01	0,38±0,01
Schizophrenia	0,28±0,03*	0,36±0,02
Multiple sclerosis	0,54±0,02	0,22±0,02*

and 0.1 ml of guinea pig complement (dried complement, packed in ampuls, diluted 1:20 with physiological saline). The samples were incubated for 2 h at 37°C in an atmosphere of air with shaking. The reaction was stopped by addition of 0.1 ml of 100% TCA. The complement and test blood serum were added to the control sample, namely homogenate incubated under the same conditions. The concentration of malonic dialdehyde (MDA) was determined by the method in [1]. Relative antioxidant activity (RAA) of the blood serum, expressed in relative units, was estimated from the difference between the MDA concentrations in the control and experimental samples.

Blood serum from 10 healthy dogs and 13 dogs with EAE, and blood serum from 10 patients with multiple sclerosis, 10 patients with schizophrenia, and also from patients with benign brain tumors, syringomyelia, osteochondrosis of the spine, myocardial infarction, psoriasis, and virus hepatitis, were investigated.

The IgG fraction was obtained by gel chromatography from the blood serum of dogs immunized with encephalitogenic emulsion, with an antibody titer of not below 1:160, by fractionation on Sephadex DEAE-50. IgG for the control experiments was obtained from blood serum of healthy dogs.

Intracisternal injection of IgG in a volume of 0.1 ml (4 mg protein) with 0.01 ml complement was given after superficial ether anesthesia to noninbred male albino rats. The rats were decapitated 24 h after the injection, the brain was frozen in liquid nitrogen, and the concentration of diene conjugates (DC) and MDA determined [8].

TABLE 3. RAA of Blood Serum (relative units) of Animals in the Course of EAE during Incubation with Homogenates of Gray and White Matter of Rat Brain (M ± m)

Exptl. conditions	Gray matter	White matter
Background	0,55±0,02	0,33±0,01
Immunization, 7th day	0,53±0,02	0,22±0,01*
EAE	0,41±0,01*	0,23±0,01*

EXPERIMENTAL RESULTS

On incubation of the blood sera of dogs with EAE with brain homogenate their RAA decreased (0.3 ± 0.06 relative units) compared with RAA of the blood sera of healthy animals (0.52 ± 0.03 relative unit). Determination of RAA of blood sera from patients with neuropsychic diseases or various somatic diseases and of healthy subjects, under the same conditions, revealed a fall in its value in patients with multiple sclerosis and schizophrenia (Table 1).

On incubation of the blood sera of dogs with EAE and of patients with multiple sclerosis and schizophrenia with liver homogenate their RAA was indistinguishable from that of the healthy subjects. Meanwhile, on incubation of blood serum of patients with virus hepatitis with liver homogenate, this parameter fell below it level in the other sera (Table 1).

The results suggested that the reduced ability of the blood serum to inhibit LPO processes in brain homogenates is due to the presence of antibrain antibodies in them. Exhaustion of the blood serum of dogs with EAE by brain tissue led to an increase in its RAA to the control values (0.48 ± 0.03 relative unit), whereas exhaustion with liver or kidney tissue did not affect these properties of the serum.

Incubation of brain homogenate with IgG isolated from the blood serum of normal dogs and dogs immunized with brain tissue (3 mg protein) confirmed this hypothesis. RAA of IgG from healthy dog blood serum was 0.21 ± 0.001 relative unit, whereas IgG from the blood serum of immunized animals was 0.12 ± 0.01 relative unit ($P < 0.001$). Incubation with liver tissue revealed no differnces between the action of "normal" and of "immune" IgG.

To study dependence of this phenomenon on the presence of complement, blood sera from patients with schizophrenia were inactivated at 56°C for 30 min and then incubated with brain homogenate. RAA of the sera before inactivation was 0.33 ± 0.03 relative unit, whereas after inactivation it was 0.46 ± 0.02 relative unit. Addition of complement to inactivated serum again led to a decrease in its ability to inhibit LPO in brain homogenates.

These experiments provide evidence that antibrain antibodies contained in the blood sera of patients with multiple sclerosis and schizophrenia and also of dogs with EAE affect the intensity of LPO processes in brain tissue. In experiments with intracisternal injection of IgG a significant increase was found in the concentration of LPO products (DC and MDA) in the rats' brain after injection of IgG isolated from blood serum of dogs immunized with brain tissue (8.1 ± 0.3 and 60.0 ± 1.2 nmoles/g tissue, respectively) compared with its value after injection of IgG from blood serium of healthy animals (6.5 ± 0.2 and 42.6 ± 1.5 nmoles/g tissue).

On the basis of these results it can be postulated that the decrease in RAA of the blood serum is due to intensification of LPO in brain homogenates under the influence of antibrain antibodies contained in the blood serum of patients with multiple sclerosis and schizophrenia and of animals with EAE. It was accoridngly decided to study this phenomenon relative to different brain structures, namely to the white matter and cerebral cortex. Blood serum from patients with schizophrenia exhibited significantly higher affinity for gray matter, and blood serum from patients with multiple sclerosis showed higher affinity for white matter (Table 2); serum of animals sensitized with brain showed affinity toward both gray and white matter (Table 3).

Investigation of the effect of blood sera on LPO in surviving brain cells can provide information on the neurotropic properties of the sera due to the presence of complement-dependent antibrain antibodies.

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ELECTROPHORETIC ANALYSIS OF LEUKOCYTE PROTEIN COMPOSITION IN DOWN'S SYNDROME

V. P. Paponov, P. S. Gromov,
L. I. Kovalev, E. G. Shcheglova,
D. M. Spitkovskii, and S. S. Shishkin

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Comparative electrophoretic analysis of polypeptide gene products in cells from patients and normal individuals in a promising approach to the study of changes in the function of the genetic apparatus of the cells in various diseases and, in particular, in those due to aneuploidy, one of the consequences of which is realized in Down's syndrome. When using this approach, as a rule investigators have turned to methods of two-dimensional electrophoresis in order to resolve the maximal possible quantity of cell proteins [5, 9, 10]. Unfortunately, previous investigations on cultures of different types of cells from human adults and embryos either revealed no changes in the protein composition of cells receiving an extra 21st chromosome [9, 10] or revealed an increased presence of only one or two proteins [5], i.e, far fewer than might be expected from the dose effect of genes expressed on the 21st chromosome [9, 10].

Using uncultured human peripheral blood cells, the writers succeeded for the first time, by one-dimensional electrophoresis, in finding a protein in leukocytes of patients with Down's syndrome that cannot be detected in normal human leukocytes [2]. In the investigation described below this feature of the leukocytes of these patients is analyzed and the causes of disagreement between the theoretically expected differences in the protein composition of normal and trisomic cells and those actually found by two-dimeniosnal electrophoresis [5, 9, 10], are discussed.

EXPERIMENTAL METHOD

Venous blood (5-15 ml) from healthy donors aged 20-40 years and from patients with Down's syndrome (8-18 years) was mixed with heparin (Richter, Hungary, 1000 IU/ml) in the ratio of 33 IU heparin to 1 ml blood, and transferred into tubes and incubated at 37°C for 2 h to sediment the erythrocytes. The leukocytes were washed off with 0.15 M NaCl and the residual erythrocytes hemolyzed in 0.035 M NaCl to produce a homogenate of leukocytes which, after freezing in water with 1 mM PMSF (phenylmethylsulfonyl fluoride), was either used immediately for electrophoresis [6], or acid extracts were prepared from it beforehand in 0.4 N HCl (°C, 30 min), and these were precipitated in 25% TCA (0°C, 12 h). These residues and residues of proteins of leukocyte homogenates not extracted with acid, were dissolved in Laemmli's protein buffer [6].

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