

ANTIGENIC DIFFERENTIATION OF THE GREY AND WHITE MATTER OF THE BRAIN IN MAN

N. I. Kuznetsova and P. N. Kosiakov

From the Laboratory of Immunology of the D. I. Ivanovskii Institute of Viro-
logy (Director — Prof. P. N. Kosiakov) of the AMN SSSR, Moscow

(Received February 4, 1958. Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)

It is known that the grey and white matter of the cerebral hemispheres differ from each other not only morphologically but also in their biochemical composition. The grey matter contains more water-soluble protein and less insoluble protein residue than the white matter [10]. The grey matter also differs from the white in its lower lipid content, and on the other hand, in the greater activity of its enzymes responsible for carbohydrate metabolism (adenosine triphosphatase, aldolase) and in other properties.

The differences in the structure of the grey and white matter of the brain and also in their biochemical composition and function gave us grounds for the belief that these two forms of tissue are characterized by qualitative antigenic differences also. However, this question has not yet been answered sufficiently convincingly in view of great difficulties in the devising of methods for reaching a solution.

Reichner and Witebsky [12] immunized rabbits with the grey and white matter of the brain of cattle, and obtained sera which reacted more intensively in the complement fixation reaction with antigens from the same brain tissue as was used for the immunization. These two forms of serum also had other differences. The sera to the white matter reacted with antigens from the brain of fishes but did not react with antigens from the brains of mammalian embryos — man and guinea pig. Conversely sera to the grey matter reacted with antigens from the embryonic brains but did not react to the brains of the fishes. On the basis of these findings these workers came to the conclusion that antigenic differences existed between the grey and white matter of the brain.

Different results were obtained by A. F. Makarchenko [9], who was unable to establish, by means of the complement fixation reaction, any antigenic differences between the grey and white matter of the brain in dogs. V. A. Korenevskaja reported that by the use of L. A. Zil'ber's method of anaphylaxis with desensitization it is possible to detect antigenic differences between the grey and white matter of the brain in man [1].

The method of selective absorption developed in our own laboratory [2-8], enabling the demonstration of specific antigens in normal and pathological tissues, was used by us in a study of the differentiation of the antigenic properties of the grey and white matter of the brain in man.

EXPERIMENTAL METHOD

Antigens from the grey matter were obtained from thin sections of the surface of the cerebral hemispheres, and antigens from the white matter — from areas of tissue from the cerebral peduncles. One g of tissue was ground in a porcelain mortar, 10 ml of a 0.85% solution of sodium chloride being gradually added. The homogeneous emulsion was centrifuged at 2000 rpm for 15 minutes; the supernatant fluid was titrated for its content of anticomplement in order to select the working dose of antigen, and it was then tested with the brain antiserum in the complement fixation reaction at 37°C.

In order to study the antigenic properties of the grey and white matter of the brain, in the first place we

TABLE 1

Immunological Activity of Brain Antisera with Antigens from the Grey and White Matter of the Human Brain

Serial no. of serum	Dilution	Antigens from tissue		
		of the grey matter	of the white matter	of the liver
79	1:20	++++	±	±
	1:40	++++	—	—
	1:80	++++	—	—
	1:160	+++	—	—
85	1:30	++++	—	—
	1:60	++(+)	—	—
	1:120	++	—	—
	1:240	+	—	—
116	1:20	++++	±	—
	1:40	++++	—	—
	1:80	+++	—	—
	1:160	++	—	—
120	1:30	+++ +	—	—
	1:60	+++	—	—
	1:120	++	—	—
	1:240	+	—	—

are capable of differentiating the antigenic properties of the grey matter of the human brain from antigens of the white matter.

Further confirmation of this was given by experiments which we carried out with the sera of rabbits immunized with the white matter of human brain. We used the white matter in view of its greater homogeneity, for it is difficult to obtain grey matter uncontaminated with white.

Rabbits were injected intraperitoneally with a 10% emulsion of white matter in doses of 2 ml on 4 occasions at intervals of 4 days between the injections. From 8-10 days after the last injection the serum was obtained and tested against saline extracts of the white and grey matter of human brain. When the experiment was performed in this way it was impossible to observe any great difference in the reaction of the sera with antigens from the white and grey matter. However, we clearly observed differences here in a specific absorption reaction. For this purpose we used fresh crude white and grey matter tissue from human brain. Before absorption, the tissues were repeatedly rinsed with physiological saline until the supernatant fluid was completely translucent. To one volume of precipitate of washed tissue was added 4 volumes of serum in a dilution of 1:10. The mixture of serum and tissue was kept in a refrigerator at +4°C for 18 hours, after which it was centrifuged at 2000 rpm for 30 minutes; the supernatant fluid was again centrifuged at 8000-10,000 rpm for 30 minutes and then tested in the complement fixation reaction with extracts of the white and grey matter of the brain and also of liver (specificity control of the serum).

The results of these investigations are shown in Table 2, which is actually one of the records from a large number of experiments of the same type.

It is seen from Table 2 that rabbit antiserum obtained to white matter of the human brain also reacted, before absorption, with antigens from the grey matter of the brain, but did not react with extracts from the liver, i. e. in these particular dilutions it showed specific activity toward the brain antigens. The quantitative complement fixation reaction showed only a slightly higher value with antigens from the white matter of the brain as compared with antigens from the grey matter. After absorption with grey matter this serum completely lost its power to react with an extract of grey matter and at the same time retained its activity toward the antigen

used sera of horses, immunized with an emulsion of the brains of mice inoculated with the viruses of tick-borne and Japanese encephalitis [11]. These sera, as shown by our preliminary investigations, contain, besides antibodies to the viruses of these forms of encephalitis, antibodies to antigens from the brain of various species of mammals, including human brain [8]. The specificity of the sera was checked by testing them simultaneously with antigens from liver tissue, prepared by the same method as the antigens from brain tissue.

EXPERIMENTAL RESULTS

The results of the investigation of the immunological activity of the brain antisera obtained from horses with antigens from the grey and white matter of human brain are shown in Table 1.

As seen from Table 1, the sera of series 79, 85, 116 and 120 gave a positive complement fixation reaction with antigens obtained from the grey matter of human brain, and at the same time did not react with antigens from the white matter. An indication of the specificity of these sera was their failure to fix complement with antigens from human liver tissue. These experiments thus showed that the sera of horses immunized with mouse brain tissue

TABLE 2

Comparative Study of the Antigenic Properties of the Grey and White Matter of the Human Brain by the Absorption Method

Sera	Dilution	Antigens from tissue		
		of the white matter	of the grey matter	of the liver
Immune rabbit serum to the white matter of human brain before absorption	1:100	++++	++++	—
	1:200	+++ (+)	+++	—
	1:400	+++	++ (+)	—
	1:800	++	+ (+)	—
Same serum after absorption with grey matter of human brain	1:20	+++	—	—
	1:40	++	—	—
	1:80	+	—	—
	1:160	±	—	—
Same serum after absorption with white matter of human brain	1:20	—	—	—
	1:40	—	—	—
	1:80	—	—	—
	1:160	—	—	—
Horse serum obtained by immunization with mouse brain tissue	1:20	±	++++	—
	1:40	—	+++	—
	1:80	—	++ (+)	—
	1:160	—	++	—

from the white matter of the brain. Conversely, as a result of treatment of the serum with white matter of the brain to which the serum had been produced, complete absorption of antibodies to antigens from white and grey matter took place.

The power of selective absorption of homologous antibodies from a serum, which is inherent in the white matter of the brain, demonstrates that antigens are present in this tissue which are absent from the grey matter.

An extract of white matter in the same experiment (see Table 2) did not react with a horse serum containing antibodies to grey matter alone.

Our investigations thus showed that the grey and white matter of the human brain have different antigenic constitutions. The grey matter contains antigens not present in the white matter, and vice versa.

The grey matter of the cerebral hemispheres in man contains antigens which are similar to the brain antigens of certain animals (mice), but these antigens are absent from the white matter of the human brain.

A comparative study of the antigenic structure of the brain in man and animals is of value towards understanding the mechanism of encephalitic phenomena sometimes observed as a result of the use of preparations from the brains of animals for purposes of treatment or prophylaxis.

SUMMARY

Saline extracts of the grey and white matter of the human brain were tested by the complement fixation reaction with various anticerebral sera.

Rabbit serum obtained after immunization by white matter of the human brain enabled precise differentiation of the antigen of the white matter from that of the grey matter after the absorption by the tissue of the grey matter. The difference in the immunological properties of the grey and white matter of the human brain was also revealed in investigation of the horse sera of horses immunized with a cerebral emulsion of mice. There was a selective reaction of these sera with the antigens of the grey matter (as compared with the antigens of the white matter). Thus, the results which were obtained show the presence of an antigenic difference between the tissues of the grey and white matter of the human brain.

LITERATURE CITED

- [1] V. A. Korenevskaja, Biull. Eksptl. Biol. i Med. 45, 2, 93-98 (1958).*
- [2] V. S. Korosteleva, Biull. Eksptl. Biol. i Med. 8, 87-93 (1957). *
- [3] V. S. Korosteleva and P. N. Kosiakov, Biull. Eksptl. Biol. i Med. 4, 83-87 (1957).*
- [4] P. N. Kosiakov, V. S. Korosteleva and N. I. Kuznetsova, Biull. Eksptl. i Med. 9, 63-65 (1955).
- [5] P. N. Kosiakov and N. I. Kuznetsova, Biull. Eksptl. Biol. i Med. 6, 49-53 (1957).*
- [6] N. I. Kuznetsova, Biull. Eksptl. Biol. i Med. 11, 52-55 (1955).
- [7] Idem, Problems of Immunology of Normal and Malignant Tissues, 156-163, Moscow (1956).* *
- [8] Idem, Voprosy Virusologii 6, 346-351 (1958).
- [9] A. F. Makarchenko, Med. Zhur. Ukrain. 20, 6, 56 (1950).
- [10] A. V. Palladin, Vestnik Akad. Nauk SSSR 10, 37-62 (1952).
- [11] I. M. Rodin and L. I. Mart'ianova, Voprosy Virusologii 3, 17-22 (1956).
- [12] H. Reichner, E. Witebsky, Ztschr. Immunforsch. Bd. 81, S. 410-432 (1934).

* See English translation.

* * In Russian.