antibodies, and gave a stronger reaction in relation to some donors with a lymphocyte suspension enriched with the B-population. In one case a serum (K-v) containing HLA-A1 antibodies and, on the basis of its rosette-inhibiting activity, belonging to group II, was absorbed by the thrombocytes of a donor who reacted with it in the lymphocytotoxic test. After absorption it lost its ability to inhibit rosette formation by T-lymphocytes. It still remained capable of inhibiting rosette formation by B-lymphocytes.

The investigations thus showed that HLA-antibodies can inhibit rosette formation by T- and B-lymphocytes of a donor containing the corresponding histocompatibility antigen. Several sera were able to inhibit rosette formation mainly by B-lymphocytes. This evidently indicates that they contain antibodies against the Ia (HLA-DR) system of antigens of human B-lymphocytes.

The inhibition of rosette formation test is a promising method for the detection of antibodies against individual lymphocyte populations.

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SYNGENEIC CEREBRAL CORTICAL TISSUE AS A STIMULATOR OF IMMUNOGENESIS IN THYMECTOMIZED MICE

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Experiments on CBA mice showed that homogenate of gray matter (cortex) of syngeneic brain contains components which restore the population of splenic T-cells in thymectomized animals and stimulate the immune response to sheep's red blood cells. Homogenate of white matter has much weaker activity; indeed it could be due to gray matter contaminating the preparation. Homogenate of syngeneic muscle tissue has no biological activity.

KEY WORDS: thymus; brain; cross-reacting antigens; stimulation of immunogenesis.

The θ-antigen of thymocytes in various species of animals is known to be present also in the brain [1, 7, 11]. This antigen is associated chiefly with the gray matter (cortex) of the brain and is virtually absent in the white matter [1, 6]. It has also been shown that extracts of thymus can stimulate the immune response in animals especially after thymectomy, and under these circumstances they functionally replace the receptors of the T-cells [2, 10, 13]. The question arises, do brain antigens have a similar effect?

The object of this investigation was to study the effect of homogenates of the cortex and white matter of syngeneic brain on the primary immune response and on the thymus-dependent lymphocyte population in thymectomized mice.

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TABLE 1. Effect of Syngeneic Homogenates of Gray and White Matter of the Brain and of Muscle on Number of AFC in Spleen of Thymectomized Mice, M ± m

Preparation tested	Number of AFC per 10^6 nucleated spleen cells after injection of homogenates			
	100 mg/day		25 mg/day	
	direct AFC	indirect AFC	direct AFC	indirect AFC
Homogenate of gray matter	51,2±3,0†	48,9±3,1†	48,6±4,8*	55,0±2,8*
of white matter	17,3±1,6	19,6±1,7	52,2±9,8	47,8±4,5
of muscle	10,5±0,1‡	10,3±0,4‡	12,0±2,5‡	9,8±2,0‡
Control 1	16,8±0,7	19,5±0,1	27,0±5,0	39,8±6,8
Control 2	8,6±0,4	9,2±0,1	11,0±2,3	9,0±1,5

Legend to Tables 1 and 2. Control 1) mice undergoing mock operation and receiving physiological saline, control 2) thymectomized animals receiving physiological saline; an asterisk and a dagger indicate that differences are significant compared with control 1 ($P < 0.05$ and 0.001 respectively), a double dagger indicates that differences are not significant compared with control 2.

TABLE 2. Effect of Syngeneic Homogenates of Gray and White Matter of the Brain and of Muscle on Serum Hemagglutinin and Hemolysin Titers in Thymectomized Mice

Antibodies	Antibody titer (M ± m)							
	physiological saline		homogenate, mg/day					
			of gray matter		of white matter		of muscle	
	control 1	control 2	100	25	100	25	100	25
Hemagglutinins	192,0±17,3	64,0±15,5	426,0±38,0†	200,0±38,8	125,0±12,5	240,0±83,3	86,6±11,2‡	52,0±12,5‡
Hemolysins	100,0±15,0	50,0±12,3	800,0±152,0*	—	80,0±14,2	—	50,0±9,7‡	—

EXPERIMENTAL METHOD

Experiments were carried out on 130 male CBA mice weighing 16-18 g. The thymus was removed from 100 animals under superficial ether anesthesia by a modified method of Galkin and Dobkin [3]. A mock operation was performed on 30 mice, i.e., all stages were carried out except removal of the thymus.

Homogenates of gray and white matter of the brain and of the thigh muscles were prepared in physiological saline and injected intraperitoneally into thymectomized animals over a period of five days in doses of 25 and 100 mg daily per animal in a volume of 0.5 ml. Injection of the tissue homogenates began on the 20th day after thymectomy. Physiological saline was injected according to a similar scheme into the control animals (thymectomized and undergoing the mock operation).

After injection of the homogenates of the corresponding tissues the animals were immunized intravenously with sheep's red blood cells, thoroughly washed with physiological saline, in a dose of 1×10^7. The mice were decapitated three days after immunization. Their sera were examined by the hemagglutination and hemolysis tests and their spleens for the presence of direct and indirect antibody-forming cells (AFC). Direct AFC were detected by the method of Jerne and Nordin [12] and indirect by the method of Dresser and Wortis [9]. To detect indirect AFC, rabbit serum (1:80) against mouse class G immunoglobulin, isolated by means of caprylic acid [15], was used. The number of direct and indirect AFC was expressed per 10^6 nucleated cells. Tests for antibodies and AFC were carried out individually on each mouse. At least five animals were used for testing each dose of different homogenates.

Antisera against the gray (cortex) and white matter of the brain were prepared by repeated subcutaneous immunization of rabbits with the corresponding material from CBA mice without Freund's adjuvant [11]. The sera were heated to 56°C for 30 min and absorbed by homogenates of white or gray matter respectively, by a homogenate of mouse liver, and by mouse and sheep's red blood cells [1] and examined in the complement fixation test (CFT) in the cold [4] with thymocytes, bone marrow cells, and homonymous antigens, and also in the cytotoxicity test [14] with thymocytes and bone marrow cells. The cytotoxic test was carried out in the presence of fresh guinea pig complement (1:3), absorbed by mouse liver and spleen homogenates, or by mouse and sheep's red blood cells in a similar manner to the absorption of complement by agarose [8]. Viable

and dying cells were differentiated with a 0.2% aqueous solution of trypan blue. No fewer than 100 cells were counted in each test. The percentage of dying cells was expressed as indices of cytotoxicity [14].

EXPERIMENTAL RESULTS

The following feature was characteristic of antigens of the gray and white matter of the mouse brain. Sera against homogenates of gray or white matter of the brain reacted after cross absorption with homonymous antigens in the CFT in dilutions of 1:320-1:640 but did not react with bone marrow cells. Serum against gray matter (after absorption with white matter) reacted with thymocytes in a dilution of 1:320, but serum against white matter (after absorption with gray matter) reacted only in a dilution of 1:20. In the complement-dependent cytotoxic test, serum against gray matter in a dilution of 1:10 caused death of 100% of thymus cells, whereas serum against white matter, on the other hand, had virtually no action on thymocytes. Neither serum reacted in the cytotoxic test with bone marrow cells.

Injection of homogenate of gray matter into thymectomized mice had a marked stimulating action on the immune response of the animals. In a dose of 100 mg/day the number of direct and indirect AFC was increased by between 2 and 4 times ($P < 0.001$), the hemagglutinin titer by between 2 and 3 times, and the hemolysin titer by 8 times ($P < 0.001$) compared with their values in animals undergoing the mock operation and receiving physiological saline (Tables 1 and 2). In a dose of 25 mg/day homogenate of gray matter also caused an increase in the number of direct and indirect AFC compared not only with the thymectomized animals, but also with those undergoing the mock operation, receiving physiological saline ($P < 0.05$), and brought the hemagglutinin titer up to the control level (in mice undergoing the mock operation) but not above it.

Homogenate of white matter of the brain had a weaker stimulating effect on the formation of AFC, hemagglutinins, and hemolysins in the thymectomized animals. Doses of 100 and 25 mg/day led to an increase in the number of direct and indirect AFC by between 2 and 4 times, and in the antibody titers by between 1.5 and 4 times compared with thymectomized mice, but caused no increase in the number of AFC or the antibody titers compared with the animals undergoing the mock operation.

Homogenate of muscle tissue affected neither the number of AFC nor the antibody titers in the thymectomized mice (Tables 1 and 2).

To analyze the action of the brain homogenates their effect on the number of T-cells in thymectomized mice was investigated. For this purpose homogenates of gray and white matter of the brain and of muscle in a dose of 25 mg/day per animal were injected intramuscularly over a period of 5 days into thymectomized mice, after which the number of T-cells in the lymph nodes and spleens was determined by the cytotoxic test with serum (1:10) against the cortical gray matter. Lymph node cells from three mice were pooled, whereas spleen cells were tested individually. At least 10-12 mice were used to test each homogenate.

Practically no decrease in the number of T-cells in the lymph nodes was found 1.5-2 months after thymectomy (the index of cytotoxicity was $70 \pm 3.2\%$ compared with $80 \pm 2.8\%$ in mice undergoing the mock operation), but their number in the spleen fell sharply ($5 \pm 1.5\%$ compared with $32 \pm 3.3\%$ in animals undergoing the mock operation). Correspondingly, injection of any of the three above-mentioned homogenates did not affect the number of T-lymphocytes in the lymph nodes ($93 \pm 1.8\%$, $90 \pm 2.1\%$, and $76 \pm 3.0\%$ respectively compared with $70 \pm 3.2\%$ in the thymectomized mice receiving physiological saline) and, conversely, it affected the number of T-cells in the spleen differentially. Homogenate of gray matter of the brain led to an increase in the number of T-cells which actually exceeded that in the animals undergoing the mock operation and receiving physiological saline ($49 \pm 3.5\%$ compared with $32 \pm 3.3\%$ in animals undergoing the mock operation; $P < 0.01$). Homogenate of white matter of the brain had a weaker stimulating effect ($28 \pm 3.2\%$ compared with $32 \pm 3.3\%$ in animals undergoing the mock operation).

Muscle tissue homogenate did not produce a significant increase in the number of T-cells compared with that in thymectomized mice receiving physiological saline ($10 \pm 2.1\%$ compared with $5 \pm 1.5\%$).

The results indicate that brain tissue, which has common antigenic components with thymocytes [1, 7, 11], can stimulate the primary immune response to sheep's red blood cells in thymectomized mice and restore the number of splenic T-cells to the control level. Homogenate of the gray matter (cortex) of the brain, where antigens common with those of the thymocytes are located [1, 6], had the strongest biological effect. Stimulation of the immune response by homogenate of gray matter was between 2 and 8 times stronger than its stimulation by homogenate of white matter. Ability to restore the thymus-dependent lymphocyte population in the spleens of the thymectomized mice was correspondingly stronger. Whereas after injection of homogenate of white matter the number of T-cells in thymectomized mice rose from 5 ± 1.5 to $28 \pm 3.2\%$, i.e., in fact up to

their level in animals undergoing the mock operation, after injection of homogenate of gray matter into thymectomized animals the number of T-cells increased to 49 ± 3.5%, i.e., above the level in the mice undergoing the mock operation. The restorative action of the brain homogenates on the lymphoid population of thymectomized animals indicates their influence on the T_1 short-living population of lymphocytes, which is sensitive to thymectomy [5].

The significantly stronger biological effect of homogenate of gray matter and the data given above for antisera against the gray and white matter are evidence that the activity is located actually in the gray matter, with which θ-antigen is predominantly associated [1, 6]. The biological activity of the homogenate of white matter can probably be explained by contamination with components of gray matter, which are extremely difficult to remove.

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