CYTOTOXICITY OF ANTISERA AGAINST BRAIN AND SPINAL CORD ANTIGENS FOR HETEROGENOUS LYMPHOCYTES

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The cytotoxic activity of rabbit antisera against brain and spinal cord antigens for mouse and guinea pig lymphocytes was investigated. None of the sera tested had any cytotoxic action on bone marrow lymphocytes, whereas sera against mouse brain and spinal cord, guinea pig brain, and myelin isolated from it were most toxic for lymphocytes of other lymphoid organs; the maximal toxic effect was found against thymocytes, it was less marked against lymph gland lymphocytes, and still less against spleen cells. The cytotoxicity of antisera against bovine spinal cord, and the myelin or basic protein isolated from it, was least of all and was the same against all the cells mentioned above; antiserum against encephalitogenic polypeptide 2c had virtually no cytotoxic activity. In its encephalitogenic properties fraction 2c considerably surpassed myelin and basic protein prepared from bovine spinal cord. Experiments with absorption of brain antiserum suggested that a cross-reacting antigen is present in the cerebral cortex. Subcutaneous injection of a relatively large dose of thymocytes (224×10^6) in Freund's complete adjuvant did not lead to the development of allergic encephalomyelitis in guinea pigs.

KEY WORDS: *Brain; spinal cord; lymphocytes; cross-reacting antigens; experimental allergic encephalomyelitis*

The existence of common antigens of the brain and thymocytes has recently been established in many species of animals [6, 8, 10, 11]. Patients' sera containing antibrain antibodies have also been shown to have a cytotoxic action on the lymphocytes of animals [1]. The study of such cross-reacting antibodies is interesting in connection with the investigation of the pathogenesis of immunopathological processes in the nervous system.

The object of this investigation was to compare the cytotoxic activity of antisera against antigens of whole brain and spinal cord, and against myelin, its basic protein, and a protein fraction (polypeptide 2c) in vitro for lymphocytes belonging to different species of animals.

EXPERIMENTAL METHOD

Myelin was obtained from brain or spinal cord by differential centrifugation in a sucrose density gradient [3]. The basic protein of myelin and its polypeptide fraction 2c were prepared by column (ion-exchange) chromatography by a modified method of Carnegie et al. [5]. Antisera against whole brain tissue of CBA mice, guinea pigs, and man and against mouse and bovine spinal cord, against myelin from bovine spinal cord, basic encephalitogenic protein, and its 2c fraction were obtained from rabbits weighing 2-2.5 kg after immunization with the corresponding preparations mixed with Freund's complete adjuvant [4]. The titers of antibodies against homologous antigen were determined in the complement fixation text in the cold [2]. All sera tested except sera against basic proteins and fraction 2c had titers of 1/320-

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TABLE 1. Cytotoxic Activity of Antisera against Whole Brain, Spinal Cord, and Its Components

Source of lymphocytes	Cytotoxic index of antisera (1:10) prepared by immunizing rabbits with antigens from							
	mouse brain	mouse spinal cord	guinea pig brain	myelin from guinea pig brain	bovine spinal cord	myelin from bovine spinal cord	BP of myelin from bovine spinal cord	fraction 2c of BP of myelin from bovine spinal cord
Thymus								
Mouse	95±1,54	67±3,31	73±3,13	61±3,31	23±2,98	22±2,93	21±2,88	4±1,39
Guinea pig	—	—	68±3,30	55±3,52	25±3,15	23±2,96	15±2,52	0
Lymph nodes:								
Mouse	66±3,34	32±3,28	60±3,46	40±3,46	21±2,86	20±2,82	18±2,70	0
Guinea pig	—	—	67±3,45	54±3,52	22±2,91	20±2,82	13±2,38	0
Spleen:								
Mouse	40±3,46	16±2,58	20±2,83	26±3,10	18±2,72	15±2,52	17±2,66	
Guinea pig	—	—	26±3,10	24±3,01	20±2,83	18±2,72	8±1,92	0
Bone marrow:								
Mouse	5±1,54	9±2,01	4±1,39	5±1,51	10±2,12	1±0,7	0	0
Guinea pig	—	—	1±0,7	3±1,21	6±1,67	0	0	0

Legend. BP) Encephalitogenic basic protein of myelin; viability of mouse lymphocytes in control series with normal serum was 80-90% in all experiments, that of guinea pig lymphocytes 70%.

1/640. Antisera against basic proteins and fraction 2c had antibody titers of 1/40. The sera were heated to 56°C for 30 min and absorbed with liver homogenates and erythrocytes [7] of the species of animal against which the cytotoxic activity of the sera were subsequently to be tested. Cytotoxic activity was tested by the method of Niederhuber and Möller [9] against the thymus, lymph gland, spleen, and bone marrow cells of the corresponding species of animal. The viability of the cells was estimated with the aid of 0.2% trypan blue.

EXPERIMENTAL RESULTS

As Table 1 shows, none of the antisera had any appreciable cytotoxic action on lymphocytes of mouse or guinea pig bone marrow. Against lymphocytes of the other lymphoid organs, the most toxic sera were those against mouse brain and spinal cord, against guinea pig brain, and against the myelin preparation from it (the indices of cytotoxicity were 40-95, 16-67, 20-73, and 24-61% respectively). The toxic effect was most marked against thymocytes, less strong in the experiments with lymph node cells, and less still against spleen cells.

Sera against bovine spinal cord and against the myelin, basic protein, and polypeptide fraction 2c obtained from it had much lower cytotoxicity (equal against cells of the thymus, lymph nodes, and spleen). Antiserum of this last type had virtually no cytotoxic action against guinea pig and mouse lymphocytes. Incidentally, in the serological tests also this serum and serum against basic protein of myelin had the lowest titers. At the same time, it must be pointed out that as regards their encephalitogenic properties, myelin protein and fraction 2c greatly surpassed the original myelin preparation: The effective dose causing the disease in 50% of inoculated animals was 1 mg for myelin, 50 µg for basic protein, and 10 µg for fraction 2c, i.e., the last two preparations were 20 and 100 times more active than myelin respectively.

To determine with what brain structures these cross-reacting antigens are connected, antiserum against human brain homogenate with a cytotoxic index of 90±2.12% against mouse thymocytes was used.

Separate batches of serum were exhausted by homogenate of human cerebral cortex or by white matter respectively. The results showed that in the first case, after absorption twice for 1 h each time (at room temperature) the cytotoxic index was lowered to 17±2.64%, and after 3 absorptions to 9±2.01%. Meanwhile absorption twice with white matter caused no appreciable decrease in the cytotoxic activity of the serum (90±2.12% before, 86±2.44% after absorption). Absorption of the serum twice with a preparation of myelin from the white matter of the brain, it will be noted, lowered its cytotoxic index, but only to 40±3.36%, i.e., by a lesser degree than treatment with homogenate of cerebral cortex.

Cross-reacting antigens are thus unquestionably present in the cerebral cortex. The fact that they could not be detected in homogenates of white matter even when the result of the experiments in which the antibrain serum was absorbed by a myelin preparation from the same white matter was positive (although not strongly so) evidently indicates, first, that cross-reacting antigens are connected with the white matter of the brain and, second, that they are screened in the native homogenate.

Since the thymocytes possessed common antigens with brain tissue and myelin, the question arises whether a clinical picture of encephalomyelitis could be induced in experimental animals by injecting them with foreign thymocytes. To answer this question, 224×10^6 mouse thymocytes were injected subcutaneously into the four foot pads of 21 guinea pigs in Freund's complete adjuvant. This number of thymocytes, according to the complement fixation test, was equivalent to 2.24 mg myelin, i.e., it was more than twice the dose of myelin required to cause the development of allergic encephalomyelitis in guinea pigs. None of the 21 animals developed the disease during the 1.5 months after inoculation. Consequently, antigens of thymocytes possessing common determinants with brain antigens are nonencephalitogenic or, alternatively, different experimental conditions and different species of animals are required in order to reveal the encephalitogenic properties of thymocytes.

LITERATURE CITED

1. M. E. Vartanyan and Kolyaskina, in: Immunology of Nervous and Mental Diseases. Abstracts of Proceedings of the All-Union Scientific Society of Neuropathologists and Psychiatrists [in Russian], Moscow (1974), p. 131.
2. V. I. Ioffe and K. M. Rozental', Zh. Mikrobiol., No. 12, 65 (1943).
3. G. V. Konovalov, Kh. Annanepesov, and V. I. Krasil'nikova, Byull. Éksp. Biol. Med., No. 5, 110 (1969).
4. P. V. Osipova and Yu. L. Zhitnukhin, Vestn. Akad. Med. Nauk SSSR, No. 1, 66 (1971).
5. P. R. Carnegie, B. Bencina, and Y. Lamoureux, Biochem. J., 105, 559 (1967).
6. J. Clagett, H.-H. Peter, J. D. Feldman, et al., J. Immunol., 110, 1085 (1973).
7. E. Feiglova, L. Pichlikova, and K. Nouza, Folia Biol. (Prague), 18, 256 (1972).
8. E. S. Golub, Cell Immunol., 2, 353 (1971).
9. J. E. Niederhuber and E. Möller, Cell Immunol., 3, 559 (1972).
10. H.-H. Peter, J. Clagett, J. D. Feldman, et al., J. Immunol., 110, 1077 (1973).
11. H. G. Thiele, R. Stark, and M. Földi, Naturwissenschaften, 59, 221 (1972).