INVESTIGATION OF THE BLAST TRANSFORMATION REACTION OF LYMPHOCYTES IN GUINEA PIGS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

P. G. Nazarov, Yu. L. Zhitnukhin, and V. I. Purin'

UDC 616.832.002-056.3-092.9-07:616.155.321.2-076.5

Experimental allergic encephalomyelitis (EAE) was induced in guinea pigs by injection of myelin from homologous brain in Freund's complete adjuvant. In the incubation period of EAE some animals showed a marked increase in the spontaneous proliferative activity of the blood lymphocytes, assessed in cultures using thymidine-H^3. With the development of clinical manifestations of the disease, the spontaneous activity of the lymphocytes reached a maximum and was exhibited in the overwhelming majority of animals. When the clinical picture of EAE was well marked, the response of the lymphocytes to stimulation by myelin preparations was observed mainly if the spontaneous mitotic activity of the cells was low or only moderately increased. Specific antigenic stimulation in vivo connected with the immunopathological process could be the cause of the increasing spontaneous activity of the lymphocytes. The state of nonspecific reactivity of the lymphocytes to phytohemagglutinin in EAE was indistinguishable from that in the control animals.

KEY WORDS: experimental allergic encephalomyelitis; lymphocytes; blast transformation.

Experimental allergic encephalomyelitis (EAE) is interesting as a model of certain demyelinating diseases of the CNS in man. Investigations [11, 4, 5] have shown that blast transformation of the lymphocytes (BTL) in response to the action of brain homogenates can be observed in EAE.

The object of the present investigation was to study spontaneous BTL and BTL in guinea pigs with EAE in experiments in vitro on the addition of specific antigen to the cell suspension.

EXPERIMENTAL METHOD

EAE was induced in noninbred guinea pigs weighing 400 g by the subcutaneous injection of 5 mg dry myelin from homologous brain in 0.3 ml Freund's complete adjuvant.

BTL was investigated in cultures of peripheral blood leukocytes ($1 \cdot 10^6$ lymphocytes in 2 ml medium No. 199 with 15% fresh autologous serum). To stimulate the lymphocytes the cultures were treated with 0.1 ml myelin in various dilutions (from 1:1 to 1:10,000) or with 0.1 ml phytohemagglutinin (PHA) (Reanal "P") in the optimal dilution (1:64-1:256). The cultures with antigen were assessed 5 days, and those with PHA 3 days after the beginning of incubation. The spontaneous BTL in control cultures (without stimulation) was assessed at the same time. Thymidine-H^3 (2 μCi) was added to the cultures 16-18 h before the end of incubation. The cells were then washed twice with 3% acetic acid to remove unincorporated isotope and were subjected to hot hydrolysis in 5% TCA by the Schmidt-Thannhauser method [3] to extract labeled DNA. The DNA digest was dried in counting flasks, the dry residue was treated with 5 ml scintillation fluid (4 g PPO, 0.05 g POPOP in 1 liter toluene), and the radioactivity was measured with a liquid

Laboratory of General Immunology, Division of Microbiology and Immunology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. Department of Propedeutics of Children's Diseases, Pediatric Medical Institute, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 2, pp. 87-90, February, 1975. Original article submitted March 20, 1974.

TABLE 1. Characteristics of Animals Studied

Time of investigation (in days)	Total	No. of animals		
		with clinical manifestations of disease	with increased spontaneous BTL	with positive BTL to myelin
7	16	0	8	4
14	12	12	12	2
21	10	6	8	2
28	9	5	7	4
56	2	0	0	1
Total . . .	49	23 (47±7%)	35 (71±7%)	13 (27±6%)

TABLE 2. Spontaneous BTL in Animals with EAE of Different Severity (in counts/min)

Group of animals	Clinical manifestations of EAE	No. of anim.	72-h culture	120-h culture
1st	Minimal or absent	26	1399 (76—13 600); $P=0,05$	699 (106—4830); $P>0,05$
2nd	Moderate or severe	23	7203 (297—46 026)	1907 (85—9157)
3rd	Control	15	167 (42—526); $P=0,01$	89 (26—239); $P<0,001$

TABLE 3. Effect of Level of Spontaneous BTL on Specific Reactivity of Lymphocytes to Myelin in Animals with EAE of Different Severity

Level of spontaneous BTL (counts/min)	Frequency of positive BLT cultures with myelin		Total
	in abs. of clin. features of EAE	in pres. of clin. features of EAE	
Under 2000	5/24	6/10	11/34
Over 2000	1/2	1/13	2/15
Total . . .	6/26	7/23	13/49

Note. Denominator gives total number of animals, numerator number of animals whose lymphocytes responded by an increase in BTL to addition of antigen to the suspension.

TABLE 4. Dependence of Reaction of Lymphocytes to PHA on Level of their Spontaneous BTL

Level of spontaneous BTL (counts/min)	Response of lymphocytes to PHA		Total
	in abs. of clin. features of EAE	in pres. of clin. features of EAE	
Under 2000	2/23	1/9	3/32
Over 2000	0/2	9/13	9/15
Total . . .	2/25	10/22	12/47

Note. Denominator gives total number of animals; numerator number of animals whose lymphocytes responded by an increase in BTL (over 64,000 counts/min) to the addition of PHA to the suspension.

scintillation counter (Nuclear Chicago Corp., Mark II). The results were expressed as the number of counts per minute. In the case of stimulation of the lymphocytes by myelin the results of the BTL reaction were assessed as positive if the amount of label incorporated at least twice as great as in the control cultures from the same animal. Statistical analysis of the results were carried out by nonparametric methods [2].

EXPERIMENTAL RESULTS

Clinical features of EAE appeared in the animals by the end of the second week after receiving the encephalitogenic mixture. The disease followed an acute course, which was accompanied by paralysis of the hind limbs, atrophy of the muscles, nutritional disturbances of the skin, and pelvic disorders.

The development of clinical manifestations of EAE was accompanied by a sharp increase in spontaneous BTL. It was observed first on the 7th day, before any signs of EAE were present (Table 1). In animals with a well-marked clinical picture spontaneous BTL was significantly higher (in 72-h cultures) than in guinea pigs with minimal signs of the disease. In both groups it was much higher than in the control group of animals (Table 2).

The level of spontaneous BTL was of decisive importance to the response of the cells to stimulation by antigen in vitro. In nearly all animals with a positive BTL to myelin, spontaneous incorporation of the label in the cultures remained at the normal level (below 526 counts/min), or was very slightly increased. With a higher level of spontaneous BTL (over 2000 counts/min) in animals with a marked clinical picture of EAE, myelin had a stimulant action on the lymphocytes significantly less frequently ($P = 0.25$; Table 3).

The reaction of the lymphocytes to PHA in the experimental group of animals was a little higher than in the control group. Incorporation of the label under the influence of PHA also depended on the spontaneous level of BTL and, unlike in experiments with the addition of myelin, the higher the spontaneous activity of the cells the more intensive the incorporation ($P < 0.025$; Table 4).

The data show that the most characteristic feature in EAE is an increase in the spontaneous BTL during culture of the lymphocytes without stimulant for 3-5 days. The spontaneous BTL level is one of the earliest features of the disease, for it rises appreciably during the incubation period, before the appearance of clinical features of EAE. With the development of symptoms the spontaneous BTL continues to rise and, in some animals, it reaches the level characteristic of the action of PHA. A matter of practical importance is that at the height of the disease an increase in the spontaneous BTL was observed in the overwhelming majority of animals, whereas in the stage of recovery the spontaneous BTL returns to normal.

The spontaneous mitotic activity of lymphocytes in culture can be regarded as the result of their stimulation by antigen in vivo. Continued proliferation of the cells in culture may also take place as a result of a small quantity of antigen present in the autologous serum. The increasing character of the spontaneous BTL is evidence that the source of specific activation of the lymphocytes is the immunopathological process developing in the CNM.

If the level of spontaneous blast transformation is high the lymphocytes do not respond to the addition of further antigen to the culture. Frequently, on the addition of antigen, DNA synthesis was not stimulated but was depressed. Under conditions of high spontaneous BTL the whole pool of antigen-reactive cells stimulated in vivo must evidently have participated already in proliferation, and for that reason the addition of antigen to the cell culture in this case would be ineffective. This may account for the observed decrease [1, 4] in the frequency of the specific blast transformation reaction in response to stimulation by brain antigens at the height of the disease, when according to the results of the present investigation spontaneous blast transformation reaches its maximal value.

LITERATURE CITED

1. A. A. Babakhin, Abstracts of Proceedings of the 4th All-Union Conference on Immunopathology [in Russian], Leningrad (1973), p. 145.
2. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973).
3. I. B. Zbarskii and S. S. Debov (Editors), The Chemistry and Biochemistry of Nucleic Acids [in Russian], Leningrad (1968).
4. I. Fowler, J. Immunol., 108, 903 (1972).
5. H. Warnatz, F. Scheiffarth, and H. Kuntz, J. Neuropath. Exp. Neurol., 29, 575 (1970).