

From Central Municipal Hospital for Infectious Diseases and
National Institute of Public Health, Budapest, Hungary.

LEUKOCYTE MIGRATION INHIBITION BY BRAIN TISSUE ANTIGENS IN ACUTE INFLAMMATORY DISEASES OF THE NERVOUS SYSTEM

By

G. NYERGES, GY. NYERGES, L. MOLNÁR & F. KOVÁCS

It was suggested decades ago that acute inflammatory diseases (AID) of the nervous system (NS), characterized by demyelination, were of an allergic nature (6, 11, 13, 22). The idea that diseases of this kind may be brought about by sensitization with nervous tissue (NT) was supported by experiences with experimental allergic encephalitis and experimental allergic neuritis in animals (16, 18, 21, 25). Findings in these experimental diseases indicated that cell-mediated delayed-type hypersensitivity to NT-antigen must be involved in their pathogenesis (1, 2, 7, 10, 12, 15, 20, 21). Accordingly several authors have been seeking specific cellular reactivity to NT-antigen in AID-s of the NS in man. Such reactivity could be demonstrated in Landry-Guillain-Barré syndrome (LGBS) patients by different methods (3, 5, 8, 9, 19, 23). Hypersensitivity to NT-antigens in other AID-s of the NS was studied in only a few cases (4, 14).

The present study was undertaken to investigate whether cellular reactivity to homologous brain antigen could be demonstrated in AID-s of the NS, with special reference to the acute disseminated encephalitides.

TABLE 1
Number of Examined Patients According to Diagnoses.

Diagnosis	Number of patients
Landry-Guillain-Barré syndrome	13
Measles encephalitis	14
Rubella encephalitis	8
Chickenpox encephalitis	1
Acute encephalitis of unknown origin	6
Lymphocytic choriomeningitis	2
Lymphocytic meningitis of unknown origin	4
Pneumococcus meningitis	1
Purulent meningitis of traumatic origin	1
Facial palsy	1
Total	51

MATERIAL AND METHODS

Patients

Fifty-one hospitalized patients were investigated. Table 1 lists the diagnoses of these patients. The control group comprised 21 persons, among them were 10 healthy subjects, while the others were suffering from non-neurologic diseases (acute enteritis, sore throat).

Antigens

Antigens were prepared from the brains (taken shortly after death) of human cadavers without neurologic diseases. Brain was cleansed of membranes and vessels, homogenized and lyophilized. From the lyophilized material two kinds of antigen were prepared: 1) Lipid-free brain tissue suspension (BTS). The lyophilized brain was extracted twice with chloroform-methanol mixture (2:1), dried and stored at 4° C. 2) Basic myelin protein (BMP) was prepared according to Kies' method (17). BTS was extracted at pH 3.5 to 2.5 and precipitated with saturated ammonium sulfate. The precipitate was dissolved in N 0.01 HCl and dialyzed. The dialyzed material was distributed in 1 ml aliquots in ampoules, lyophilized, and stored at 4° C. The encephalitogenic activity of the BMP was determined in animal experiments. One part of BMP dissolved in saline was mixed with one part of Freund's complete adjuvant (Difco). Institute-bred guinea-pigs of 350-400 g were inoculated with 0.2 ml of this mixture intracutaneously. Two to three weeks after being injected with 10 µg BMP, 5 out of 7 animals exhibited symptoms of encephalitis, and 3 of them died. Four out of six animals inoculated with 3 µg BMP developed encephalitis; none of them died, however. Histological examination of the brains of the dis-

ceased animals proved the presence of lesions characteristic of experimental allergic encephalitis.

Leukocyte migration inhibition (LMI)

Tests were carried out according to the method developed by Søborg & Bendixen (24). Venous blood was allowed to sediment in a heparin-dextran mixture at 37° C for 1 hour. Leukocytes were washed three times in Hanks' BSS. The washed sediment was resuspended in P 199 medium containing 10 per cent horse serum ("culture medium"), and transferred into capillary tubes. Culture chambers were constructed from plastic rings 10 mm in diameter attached to microscopic slides with silicone wax. After a short centrifugation the capillaries were cut at the cell-fluid interface and attached to the bottom of the chamber with silicone wax. The chambers were filled with the culture medium with or without antigen and covered with cover-slips. For testing with BMP, 100 µg BMP was dissolved in 1 ml culture medium. For testing with BTS, 1 mg of the lyophilized lipid-free brain tissue was ground in 10 ml saline and filtered through paper. From the filtrate 0.1 ml was added to 0.9 ml culture medium. Each migration experiment was carried out in triplicate, usually with both BMP and BTS as antigens, while 3 chambers without antigen served as controls. Chambers were incubated at 37° C for 18 hours. Migration areas were projected onto paper, cut out and weighed on an analytical balance. The migration index (MI) was calculated according to the formula

$$MI = \frac{\text{migration in antigen-containing culture}}{\text{migration in antigen-free culture}}$$

MI-s less than 0.8 and more than 1.2 proved significant when evaluated with Student's t-test.

RESULTS

Significance of the Antigen used in the LMI Test

Fig. 1 shows 189 MI values, i.e. the results of all the tests carried out in the course of the study.

Fifty-one patients with AID of the NS were tested with the BTS in 78 examinations altogether. An MI of less than 0.8 was found in 35 tests (44.8 per cent). Fifty of these patients were tested with BMP as antigen in 70 examinations altogether. An MI of less than 0.8 was found in 39 tests (55.7 per cent). Neither BTS nor BMP induced significant stimulation of the migration.

Twenty-one control subjects were each tested with the BTS

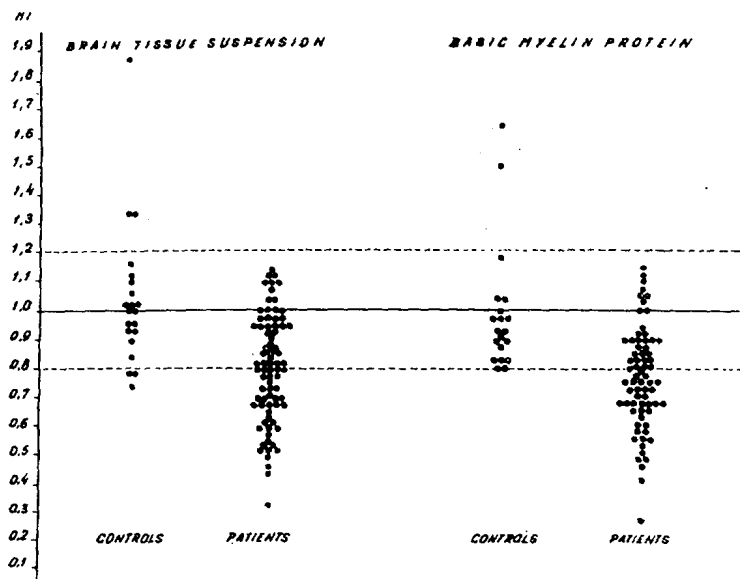


Fig. 1.

MI values observed in all the LMI tests carried out in the course of the study.

on one occasion. In 3 cases MI-s of less than 0.8, and in another 3 cases MI-s of more than 1.2 were found. Twenty of these control subjects were each tested with BMP on one occasion. No MI of less than 0.8 was found; stimulation (MI over 1.2) occurred in 2 cases.

Patients were tested with both antigens simultaneously on 70 occasions altogether (Table 2). Results were identical in 52 tests. Significant inhibition of migration was induced in 5 tests only by BTS, and in 13 tests only by BMP.

The frequency of the LMI test positivity in AID of the NS. Table 3 presents the MI values found in different forms of AID of the NS. Except for 3 cases where LMI tests were carried out with BTS only, MI-s refer to the results obtained

TABLE 2

Comparison between Brain Tissue Suspension (BTS)- and Basic Myelin Protein (BMP)-Antigens used in the Leukocyte Migration Tests. Results of 70 Investigations on 51 Patients with Different Acute Inflammatory Diseases of the Nervous System.

Result of the leukocyte migration test with		Number of investigations
BTS	BMP	
antigens		
+	+	26
—**	—	26
+	—	5
—	+	13

* migration index < 0.8

** migration index > 0.8

with BMP antigen, since this was found more suitable for testing than BTS (see above).

When looking for the frequency of LMI positivity in different diseases the following conclusions can be stated. An MI of less than 0.8 was found at least once during the course of the disease in 7 out of 13 patients with LGBS (53.8 per cent); in 9 out of 14 patients with measles encephalitis (64.4 per cent); in 5 out of 8 patients with rubella encephalitis (62.2 per cent); in 5 out of 6 patients with encephalitis of unknown origin (83.3 per cent); and in 4 out of 9 patients with other NS diseases (44.4 per cent). No inhibition of migration was found in the single case of varicella encephalitis. Since the number of cases per group was rather small, and moreover repetitions of the tests were not uniformly done, differences in the frequency of the LMI positivity cannot be evaluated.

As to the changes in the MI values during the course of the LGBS, very scanty information could be obtained. Two patients could be tested in the first week of the disease; MI values were found to be normal for both. One of them became positive later on (patient No. 13). It may be of significance

TABLE 3

Results of Leukocyte Migration Tests with Basic Myelin Protein in Acute Inflammatory Diseases of the Nervous System.

Patient No.	Age (years)	Diagnosis	Aetiology	MI values					
				1-7	8-14	15-21	22-28	29-60	> 60
				days after the onset of the disease					
1.	25	LGBS	?		1.0	0.75	0.25	1.13	
2.	15	"	?			0.51		1.05	0.75
3.	24	"	?		0.48			died	
4.	1	"	?		0.86				
5.	26	"	?		0.83				
6.	67	"	?		0.69		died		
7.	2	"	?				0.56		0.87
8.	2	"	?					0.93	
9.	21	"	?		0.68		0.57		
10.	35	"	?			0.89			
11.	7	"	measles	1.0*		1.06			
12.	21	"	SLE						1.10
13.	45	"	?	0.9		0.48			
14.	10	ADE	measles	0.68		0.60		0.96*	
15.	7	"	"	0.71					
16.	9	"	"		0.79				
17.	13	"	"	0.64	0.67				0.82
18.	12	"	"	0.82	0.45				0.91**
19.	17	"	"		0.72				
20.	10	"	"	0.66	0.89**				
21.	6	"	"	0.60*					
22.	12	"	"	0.39		0.77**			
23.	7	"	"		1.1				
24.	4	"	"	0.89					
25.	6	"	"	0.83					
26.	13	"	"	0.86					
27.	7	"	"	1.12					
28.	6	"	rubella		0.55**		0.67**		0.68**
29.	8	"	"		0.87				
30.	5	"	"		0.71				
31.	9	"	"	0.78			0.66**		
32.	4	"	"	0.72			0.82		
33.	6	"	"	0.65					
34.	6	"	"	0.86					
35.	4	"	"		0.90				
36.	5	"	varicella	1.08					

TABLE 3 (cont.)

Patient No.	Age (years)	Diagnosis	Aetiology	MI values					
				1-7	8-14	15-21	22-28	29-60	> 60
				days after the onset of the disease					
37.	9	ADE	?			0.72*	0.89		
38.	9	"	?	1.01					
39.	12	"	?	0.54				0.92	
40.	13	"	?		0.76				
41.	12	"	?		0.68				
42.	7	"	?	0.66				0.75**	
43.	15	meningit.	LCM	0.82					
44.	10	"	LCM	0.85					
45.	27	mening.ly.	?					0.58	
46.	22	"	?				0.84		
47.	12	"	?		0.69	0.68			
48.	12	"	?	0.58					
49.	11	meningit.	pneumococ.	0.80					
50.	15	"	traumatic					0.63	
51.	16	facial palsy	?		0.83				

Abbreviations: LGBS: Landry-Guillain-Barré syndrome; ADE: Acute disseminated encephalomyelitis; SLE: Systemic lupus erythematosus; LCM: Lymphocytic choriomeningitis virus; mening. ly.: meningitis lymphocytica.

Symbols:  period of clinical symptoms.

* tested with brain tissue suspension.

** EEG-changes still present.

that rather low MI values were found in two fatal cases (Patients No. 3 and No. 6).

Data relating to patients with acute disseminated encephalitis give some more information. Nineteen patients were tested in the first week of the disease, mainly between the first and third days. MI less than 0.8 was found in 11 of them. Patient No. 18, who had a normal MI value on the first day, became positive by the second week, while the MI value returned to normal during convalescence. Thirteen patients were tested after clinical recovery. Eight of them had normal MI values by this time. In the 5 cases with MI-s of less than 0.8, EEG changes were still present.

DISCUSSION

By means of the LMI test we demonstrated cellular reactivity to brain tissue antigen in 30 out of 51 patients with AID of the NS; i.e. in a rather high proportion of the patients tested.

We found BMP superior to the relatively crude BTS preparation for two reasons: 1) BMP detected hypersensitivity in neurological patients more frequently than BTS did. 2) BMP induced no inhibition of migration in any of the normal controls, while significant inhibition was found in 3 out of 21 controls tested with BTS. Thus tests carried out with BMP proved more sensitive and more specific than those with BTS. This observation, although unusual with *in vitro* tests, may be explained by the difference between the physical states of the antigens. BMP dissolves in the culture medium quickly and completely so that the BMP concentration could be held constant over the whole period of the study. On the other hand, BTS does not dissolve easily; therefore the amount and size of the dissolved and suspended particles may not have been sufficiently standardized. As a result, the concentration in the BTS-containing chambers of the different brain tissue antigens, including the encephalitogenic protein, might have varied from test to test.

Our findings on the cellular reactivity to NT-antigens in LGBS patients agree with those of several authors, though most of the data published refer to antigens prepared from peripheral nervous tissue (3, 5, 8, 9, 19, 23). Apart from the few cases cited in the introduction, however, we found no data referring to patients with acute disseminated encephalitis. We observed significant BMP-induced migration inhibition in 19 out of 29 patients with acute disseminated encephalitis, i.e. in a considerable proportion of the patients. Thus, acute disseminated encephalitis does not differ much from LGBS in this respect. These observations, however, give no reliable answer to the question as to whether hypersensitivity to BMP would be an important factor in the pathogenesis of the acute disseminated encephalitis. We found significant MI values in 11

out of 19 patients tested in the very first days of the disease; yet hypersensitivity usually disappeared after complete recovery. These facts speak for the pathogenetical role of the hypersensitivity to BMP. On the other hand, significant MI values were also observed in some patients with NS diseases that have not been considered to be of an allergic nature (Table 3. Patients No. 43 to 51). Here we refer to Rocklin et al. (23), who found lymphocytes of patients with cerebrovascular accidents sensitive to NT antigen. Thus the importance of the hypersensitivity in the development of acute inflammatory diseases of the NS is far from being clear and could be defined in carefully planned follow-up investigations only. Further studies to this end are under way.

SUMMARY

Fifty-one patients with acute inflammatory diseases of the nervous system, and 21 control subjects were investigated for cellular reactivity to homologous brain tissue antigens by means of the leukocyte migration test. A lipid-free brain tissue suspension and basic myelin protein were used as antigens. Leukocyte migration tests with basic myelin protein proved more sensitive and more specific than those with brain tissue suspension. Significant migration inhibition induced by basic myelin protein was found in 7 out of 13 patients with Landry-Guillain-Barré syndrome; and in 19 out of 29 patients with acute nervous system diseases, i.e. meningitis and facial nerve palsy. Hypersensitivity to basic myelin protein was demonstrated in 11 out of 19 acute disseminated encephalitis patients tested in the first days of the disease.

ACKNOWLEDGEMENTS

We thank Miss Olga Funk, Miss Márta Antóny and Mrs Piroska Balcsik for their competent technical assistance. We thank Dr. Dénes Karasszon for the histological examinations of the experimental animals.

REFERENCES

1. Arnason, B. G., Jankovic, B. D., Waksman, B. H. & Wennerstein, G. (1962): Role of the thymus in immune reactions in rats. II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and the circulating small lymphocytes. *J. exp. Med.* *116*, 1177-1186.
2. Arnason, B. G., Wincler, G. F. & Hadler, N. M. (1969): Cell mediated demyelination of peripheral nerve in tissue culture. *Lab. Invest.* *21*, 1-10.
3. Behan, P. O., Behan, W. H. H., Feldman, R. G. & Kies, M. W. (1972): Cell mediated hypersensitivity to neural antigens. (Occurrence in human patients and non-human primates with neurological diseases). *Arch. Neurol. (Chic.)* *27*, 145-152.
4. Behan, P. O., Geschwind, N., Lamarche, J. B., Lisak, R. P. & Kies, M. W. (1968): Delayed hypersensitivity to encephalitogenic protein in disseminated encephalomyelitis. *Lancet* *II*, 1009-1011.
5. Behan, P. O., Lamarche, J. B., Feldman, R. G. & Sheremata, W. A. (1970): Lymphocyte transformation in the Guillain-Barré syndrome. *Lancet* *I*, 421.
6. Bogaert, van L. (1933): Les manifestations nerveuses au cours des maladies éruptives (varicelle, rougeole, scarlatine). *Rev. neurol.* *1*, 150-167.
7. Bornstein, M. B. & Appel, S. H. (1965): Tissue culture studies of demyelination. *Ann. N. Y. Acad. Sci.* *122*, 280-286.
8. Castaigne, P., Berthaux, P., Brunet, P., Moulias, R., Goust, J.-M. & Delrieu, F. (1972): Polyradiculonevrites inflammatoires et immunité cellulaire. Étude de la réponse immunitaire cellulaire envers des antigènes de nerf périphérique par le test de migration des leucocytes. *Nouv. Presse méd.* *1*, 2445-2449.
9. Currie, S. & Knowles, M. (1971): Lymphocyte transformation in the Guillain-Barré syndrome. *Brain* *94*, 109-116.
10. David, J. R. & Paterson, P. Y. (1965): In vitro demonstration of cellular hypersensitivity in allergic encephalomyelitis. *J. exp. Med.* *122*, 1161-1171.
11. Finley, K. H. (1938): Pathogenesis of encephalitis occurring with vaccination, variola and measles. *Arch. Neurol. Psychiat. (Chic.)* *39*, 1047-1054.
12. Freund, J. & Lipton, M. M. (1955): Experimental allergic encephalomyelitis after the excision of the injection site of antigen-adjuvant emulsion. *J. Immunol.* *75*, 454-459.
13. Glanzmann, F. L. (1927): Die nervösen Komplikationen von Varizellen, Variola Vakzine. *Schweiz. med. Wschr.* *57*, 145-154.
14. Hashem, N. & Barr, M. L. (1963): Mitogenic effect of rabies vaccine on culture of lymphocytes in diseases of the nervous system. *Lancet* *II*, 1029-1030.
15. Jankovic, B. D. & Isvaneski, M. (1963): Experimental allergic encephalomyelitis in thymectomized, bursectomized and normal chickens. *Int. Arch. Allergy* *23*, 188-206.
16. Kabat, E. A., Wolf, A. & Bezer, A. E. (1947): Rapid production of acute

- disseminated encephalomyelitis in rhesus monkeys by injections of heterologous and homologous brain tissue with adjuvants. *J. exp. Med.* 85, 117-130.
17. Kies, M. W. (1965): Chemical studies on encephalitogenic protein from guinea-pig brain. *Ann. N. Y. Acad. Sci.* 122, 161-169.
 18. Kies, M. W. & Alvord, E. C. Jr. (1959): "Allergic" encephalomyelitis: proceedings of a symposium on experimental "allergic" encephalomyelitis and its relation to other diseases of man and animals. Charles C. Thomas, Springfield, Illinois.
 19. Knowles, M., Saunders, M., Currie, S., Walton, J. N. & Field, E. J. (1969): Lymphocyte transformation in the Guillain-Barré syndrome. *Lancet II*, 1168-1170.
 20. Paterson, P. Y. (1960): Transfer of allergic encephalomyelitis in rats by means of lymph node cells. *J. exp. Med.* 111, 119-136.
 21. Paterson, P. Y. (1966): Experimental allergic encephalomyelitis and autoimmune disease. *Advanc. Immunol.* 5, 131-208.
 22. Pette, H. (1942): Die akut entzündlichen Erkrankungen des Nervensystems. G. Thieme, Leipzig.
 23. Rocklin, R. E., Sheremata, W. A., Feldman, R. G., Kies, M. W. & David, J. R. (1971): Cellular response in Guillain-Barré syndrome and multiple sclerosis. *New Engl. J. Med.* 284, 803-808.
 24. Søborg, M. & Bendixen, G. (1967): Human leukocyte migration as a parameter of delayed hypersensitivity. *Acta med. Scand.* 181, 247-255.
 25. Waksman, B. H. & Adams, R. D. (1955): Allergic neuritis: an experimental disease of rabbits induced by injection of peripheral nervous tissue and adjuvants. *J. exp. Med.* 102, 213-235.

Author's address:

Dr. G. Nyerges,
Gyáli ut 5-7,
1097 Budapest,
Hungary.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.