Multiple Sclerosis: Lymphocyte Transformation by Basic Protein from Bovine Spinal Cord

There have been several recent reports that lymphocytes from patients with multiple sclerosis are transformed in culture by cerebrospinal fluid and by encephalitogenic proteins[1–5]. There have also been reports challenging these results[6–10]. We are currently studying this phenomenon and report here the results of two preliminary experiments.

Volunteer blood donors were normal individuals from the laboratory, patients of private physicians and patients from the rehabilitation ward of a local hospital. Heparinized samples (20 ml) were processed by slight modifications of the technique described by HASTINGS et al.[11] and HIRSCHHORN et al.[12]. Culture fluid was Eagles minimal medium supplemented with glutamine (2 µg/ml), streptomycin (50 µg/ml), neomycin (75 µg/ml) and 20% fetal calf serum. Culture vessels were 10 ml vials sealed with silicone rubber stoppers. Each culture had a volume of 3 ml and contained ca. 3×10^6 lymphocytes (most other leucocytes were removed by prior incubation on glass for 1 h). After incubation at 37 °C for 70 h, 0.1 ml of a 15.5 µg/ml solution of vinblastine sulfate was added, and incubation was continued for 2 h. Cells, harvested, fixed, and dried on cover slips as described by HASTINGS et al.[11], were stained by the Giemsa or May-Grunwald-Giemsa method.

Microscopic examination revealed very few mitoses because of the short incubation period. To avoid the possible ambiguity of deciding whether a cell was a lymphocyte or an early blastoid form, we chose to carry out differential counts based on size only. Cells were judged to be small if they were smaller than 8.8 µ in diameter; other cells were judged to be large. About 400 cells were counted on each slide and 4 slides were counted for a total of about 1600 cells, except in a few instances in the first experiment when only 2 slides were counted. Slide to slide variation was usually within the expected binomial limits, and the percentages reported in the Tables were calculated from the pooled counts.

Defining blastogenesis operationally in terms of cell size has the advantage of making the cell counts more objective. Unfortunately, the difference between stimulated and control cultures is then biased toward being smaller than true value, since the former contain more blastoid cells which are slightly too small for the definition than do the latter. Encephalitogenic crude basic protein used as antigen was derived from bovine spinal cord and has been described previously[13].

In the first experiment, a simple question was posed, namely, do lymphocytes from any multiple sclerosis patient show transformation in culture with a spinal cord antigen? Lymphocytes from 4 normal individuals and 7 patients with multiple sclerosis were cultured, and the results are shown in Table I. The answer to the question was unequivocal; lymphocytes from some multiple sclerosis patients are transformed, and in this particular group of patients all responded. The results demonstrate that work with bovine antigens can be applied to the human problem, and they are consistent with and fortify the hypothesis that multiple sclerosis is an immunological disease.

The obvious possibility that this test might have potential as a diagnostic aid was examined in one additional experiment. Blood samples were processed 'blind' from patients with various disorders, and the results are reported in Table II. In the multiple sclerosis group, lymphocytes from some patients responded, some did not. In the non-multiple sclerosis group there were also responders, and these fell into 2 sub-groups, those with

Table I. Blastogenic effect of bovine spinal cord antigen on human blood lymphocytes from normals and from patients with multiple sclerosis

Donor	Large cells + mitoses		
	With antigen		Without antigen
	(µg/ml)	(%)	(%)
Normals			
W.T.	25	0.7	1.1
R.S.	25	1.5	0.6
J.B.	25	1.5	0.7
	50	1.2	1.1
W.O.	25	1.5	1.1
Multiple sclerosis patients			
M.H.	3.3	5.6[a]	0.8
	10	5.5[a]	
	30	5.8[a]	
	90	5.4[a]	
C.N.	5	34.3[a]	16.6
	50	42.1[a]	
K.H.	5	3.4[a]	0.7
	50	10.3[a]	
A.T.	5	9.9[a]	2.5
	50	8.0[a]	
E.G.	5	30.2[a]	16.7
	50	19.4	
S.	5	31.2[a]	10.6
	50	3.0[a]	
R.B.	5	5.5[a]	1.5
	50	8.8[a]	

[a] Chi-square test, $P < 0.001$.

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Table II. Blastogenic effect of bovine spinal cord antigen on human blood lymphocytes from a variety of hospital patients

Sex	Age	Reason for confinement	Percentage large cells + mitoses		
			No antigen	With antigen (µg/ml) 5	50
♂	34	Multiple sclerosis	2.63	5.59[a]	7.69[a]
♂	19	Multiple sclerosis	1.60	3.27[b]	3.91[a]
♂	46	Multiple sclerosis	0.81	1.00	3.33[a]
♂	55	Advanced multiple sclerosis	1.18	1.69	2.12
♀	53	Multiple sclerosis? Parkinsonism?	0.69	0.63	0.94
♀	36	Advanced multiple sclerosis	1.00	0.87	1.51
♂	50	Multiple sclerosis	1.62	3.37[b]	2.19
♂	59	Tuberculosis, diabetes	2.37	2.06	2.43
♂	63	Leukemia	1.19	1.67	1.50
♀	44	Pain, unknown etiology	2.31	2.81	1.24
♂	49	Open heart surgery	1.61	1.50	2.62
♀	50	Cerebrovascular accident	1.12	1.25	2.75[b]
♂	55	Cord lesion (cyst? syringomyelia?)	0.44	–	2.25[a]
♀	83	Fracture	1.06	3.13[a]	2.87[a]
♀	77	Osteoarthritis	1.31	1.81	5.24[a]
♂	81	Tumor of spine?	1.81	5.18[a]	4.62[a]

[a] Chi-square test compared to control, $P < 0.001$. [b] Chi-square test, $0.001 < P < 0.01$.

central nervous lesions and those in their eighth or ninth decade of life. The latter raises an interesting geriatric question which demands further investigation.

With such limited data one is unwilling to generalize broadly, but our results are fully consistent with those of HUGHES et al.[5] who reported that lymphocytes from patients with multiple sclerosis are transformed when cultured in the presence of an encephalitogenic protein, not dramatically but to a greater extent than those from patients with other neurological disease and those from normals[14].

Résumé. On a constaté de la blastogenèse dans une culture de protéine encéphalitogène de moelle épinière bovin faite avec des lymphocytes de malades atteints de sclérose en plaques ou présentant des lésions du système nerveaux central, ou encore agés de plus de 70 ans.

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Comparative Study of the Ultrastructure and Hormonal Content of the Proximal and Distal Stumps of the Transected Neurosecretory Hypothalamo-Hypophysial System[1]

The hypothalamus is linked to the neural lobe of the hypophysis via the hypothalamo-neurohypophysial tract. Within the axons of this tract neurosecretory material – the polypeptide hormones oxytocin and vasopressin, and a carrier protein (neurophysin) – which can histologically be demonstrated by a positive Gomori reaction is carried by a proximo-distal axoplasmic flow from the synthesizing perikarya into the neural lobe of the hypophysis where it is stored and released[2,3].

After transection of the hypothalamo-neurohypophysial tract an increased amount of Gomori positive substance is not only observed in the proximal stump but also in the *distal stump*[4–8]. As it is very unlikely that neurosecretory material is produced within disconnected axons and as the light microscope alone could not give a satisfactory explanation for the observed phenomenon, the present studies were carried out.

For the ultrastructural studies 3 grass frogs (*Rana pipiens*) were sacrificed at each of the following time periods following transection of the proximal neurohypophysis[8]: 6, 12, 24, 36, 48 h, 6 and 9 days; 7 sham operated animals served as controls. After decapitation, fixation of the stumps was achieved by direct application and ventricular perfusion of a threefold aldehyde mixture[9] in which the dissected tissue remained for 2 h; this procedure was followed by 2 h postfixation in 1% osmium tetroxide (pH 7.2), en bloc staining with uranyl acetate[10], embedding into araldite 502, and staining of the sections with lead citrate[11].

The pharmacological extracts were obtained from 2 groups of 6 animals (controls and 6 h), 2 groups of 7 and 6 animals (1 day), 2 groups of 8 and 7 animals (2 days), 1 group of 6 animals (9 days), and 1 group of 3 animals (15 days) by pooling and homogenizing in 0.25% acetic acid at 0 °C the proximal (including the hypothalamus caudal to the optic chiasm) and the distal stump (including the median eminence) separately for each group. Further treatment of the homogenates included immersion in boiling water for 3 min and subsequent centrifugation at 3000 rpm for 10 min. The bioassays[12] [(2 + 2)] assay

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