Preliminary Communications

Lymphocyte Transformation in Multiple Sclerosis*

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Summary: Lymphocytes in cultures of peripheral blood cells from patients with multiple sclerosis showed significant blastogenic transformation in the presence of human brain extract, encephalitogenic myelin basic protein, or human cerebrospinal fluid, but not in the presence of kidney extract. There was no correlation between the degree of activity of the patients' disease and the percentage transformation of their lymphocytes.

Introduction

Multiple sclerosis may be a disorder related to cellular hypersensitivity to myelin basic protein. While not the primary aetiological cause, delayed hypersensitivity assumes importance as a possible pathogenic mechanism, strenuous modification of which could offer some therapeutic advantage.

Lymphocyte transformation in vitro in the presence of specific antigen while related to the secondary production of immunoglobulins has been shown to correlate with delayed hypersensitivity (Oppenheim, 1968). Benezra *et al.* (1969) found that lymphocyte transformation occurred not only with delayed hypersensitivity but also with the immediate type, and suggested that these two cellular functions were due to two different cell populations. Investigators of the blastogenic transformation of peripheral lymphocytes from multiple sclerosis patients, when cultured alone or with various brain antigens, reported disparate results. Among these were:

(a) Spontaneous transformation in autologous serum as measured by labelled thymidine uptake. Hughes *et al.* (1968), using the scintillation counting technique, found that the mean counts per minute were below 1,000 in multiple sclerosis patients and were 3,000 in normals; and Brody *et al.* (1968), using autoradiographic studies, found the range percentage of labelled cells was 0.0 to 0.1 in multiple sclerosis patients and 0.0 to 0.2 in controls.

(b) Blastogenic response to cerebrospinal fluid. Fowler *et al.* (1966) found this greater in multiple sclerosis patients than in controls as measured by morphological changes, while Brody *et al.* found there was no increase as measured by autoradiographic techniques.

(c) Blastogenic response to myelin basic protein (encephalitogenic factor). According to Hughes *et al.* (1968) this was greater in multiple sclerosis patients than in normal controls as measured by labelled thymidine uptake and scintillation techniques, while Behan *et al.* (1968) found there was no increase as measured by morphological and autoradiographic techniques.

These differences are difficult to reconcile. Conditions of experiments are not always the same. Patients, preparation of antigens, concentration of cells, techniques, and time period of cultures may vary. Individually and collectively these may account for the variations. Some of these investigators also used a cell population of pure lymphocytes, which may account for an erratic and limited blastogenic response compared with the lymphocyte transformation in peripheral blood leucocyte cultures.

Macrophage processing of antigen is necessary for blastogenic response to antigen (Hersh and Harris, 1968).

Lymphocyte-macrophage interaction has been found in cultures of human leucocytes, especially those undergoing blastogenic response to antigen (Bartfeld and Juliar, 1964). Hersh and Harris (1968) showed that purified lymphocytes had reduced or no blastogenic response to antigen when compared with unseparated leucocytes, and cultures of purified lymphocytes on autochthonous macrophages restored their response. Also, as the concentration of purified lymphocytes was increased their response to antigen improved slightly as there was more chance of lymphocytes making contact with the few macrophages remaining after separation.

We report studies of the cellular reactivity of multiple sclerosis patients as shown by transformation of lymphocytes present in cultures of their peripheral white blood cells with various brain antigens.

Subjects and Methods

The patients studied were from the neurology and rehabilitation services of Manhattan Veterans Administration Hospital, Bellevue Hospital, and St. Vincent's Hospital and Medical Center. In 22 patients the diagnosis was multiple sclerosis and in one patient it was Devic's disease, a variant of multiple sclerosis; 12 of the 23 were considered to be in the active and progressive stages and 11 in the stable and burnt-out stages. The diagnoses of the 18 control patients were: 12 cerebrovascular accident, one Brown-Séquard syndrome following gunshot wound to spine, one lumbar radiculopathy, one myelitis following radiotherapy nine months previously for Hodgkin's disease, one brain tumour, one cerebellar degeneration, and one retrobulbar neuritis associated with exophthalmos. In addition there were three patients with amyotrophic lateral sclerosis and also one patient with Wilson's disease, who had been diagnosed for five years as having progressive multiple sclerosis.

Laboratory Methods

Antigen Preparations.—Human brain extract: white matter was minced and homogenized as 20% solution by wet weight in TCM-199 and centrifuged at 2,000 r.p.m. for 15 minutes, the supernatant being used. Human kidney extract was prepared as human brain. Homologous pooled and non-pooled human cerebrospinal fluid was obtained from diagnostic lumbar taps; these were non-infected, but were filtered before use.

Peripheral Blood Cultures.—Plasma containing white blood cells was separated from heparinized venous blood. Volumes were adjusted so that 3 ml. of culture medium contained 1.5×10^6 cells. The medium consisted of 80% TCM-199 and 20% autologous plasma with glutamine but no antibiotics.

Antigens were added to replicate culture tubes as follows: 0.2 ml. of human brain extract, 0.2 ml. of cerebrospinal fluid, 0.2 ml. of human kidney extract, 25 μ g. of human myelin basic protein furnished by Dr. M. W. Kies. Control cultures without antigens and pokeweed mitogen 0.025 mg./ml. were also

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studied. After incubation for four days at 37°C. the cells were harvested by centrifugation and washed with balanced salt solution suspended in Carnoy's solution, and smears were made on cover-glasses. These were stained with Jenner-Giesma stain. The preparations were studied under high power ($\times 1,000$) for blastogenic transformation, carefully differentiating lymphoblasts from macrophages. Then 1,000-2,000 cells were counted and transformation was scored as the number of lymphoblasts per total number of mononuclear cells.

Results

The Table lists the percentage lymphocyte blastogenic transformation of multiple sclerosis, amyotrophic lateral sclerosis and control patients. There was no significant difference of response of these groups in spontaneous transformation or to pokeweed mitogen and to human kidney extract. Significant differences were found in the response of multiple sclerosis patients to human brain extract ($P < 0.001$), human cerebrospinal fluid ($P < 0.001$) and myelin basic protein ($P < 0.002$) compared with the control group. There were also significant differences in the response of multiple sclerosis patients to

Lymphocyte Transformation in Peripheral Leucocyte Cultures of Multiple Sclerosis (M.S.), Amyotrophic Lateral Sclerosis (A.L.S.) and Control Patients (Cerebrovascular Accident and Other Diseases)

Antigen	Transformation % (Mean $\pm$ S.E. of Mean)		
	M.S.	A.L.S.	Controls
None	5.3 $\pm$ 1.0 (23)	3.6 $\pm$ 0.4 (3)	4.0 $\pm$ 0.6 (18)*
Pokeweed mitogen	30.7 $\pm$ 1.4 (23)	30.7 $\pm$ 5.9 (3)	27.3 $\pm$ 2.4 (18)*
Human:			
Brain extract	19.3 $\pm$ 1.7 (22)	4.3 $\pm$ 1.2 (3)	5.8 $\pm$ 1.5 (17)†
Cerebrospinal fluid	16.9 $\pm$ 1.3 (22)	4.0 $\pm$ 0.0 (3)	6.2 $\pm$ 1.7 (17)†
Myelin basic protein	16.7 $\pm$ 3.1 (6)		5.2 $\pm$ 1.5 (5)‡
Kidney extract	5.9 $\pm$ 0.6 (21)	3.7 $\pm$ 1.3 (3)	5.2 $\pm$ 0.7 (14)§

Numbers in parentheses refer to number of patients.

*12 cerebrovascular accident, 1 Brown-Séquard syndrome following gun-shot wound, 1 lumbar radiculopathy, 1 myelitis following radiotherapy for Hodgkin's disease, 1 brain tumour, 1 cerebellar degeneration, 1 retrobulbar neuritis possibly associated with exophthalmos.

†As in * but patient with Brown-Séquard syndrome not studied.

‡2 cerebrovascular accident, 1 brain tumour, 1 cerebellar degeneration, 1 retrobulbar neuritis.

§As in * but 3 cerebrovascular accident patients and patient with Brown-Séquard syndrome not studied.

human brain extract ($P < 0.01$) and human cerebrospinal fluid ($P < 0.01$) compared with amyotrophic lateral sclerosis patients whose response was essentially as in the larger control group. The one case of Wilson's disease that had been diagnosed as progressive multiple sclerosis for five years showed transformation equal to that of multiple sclerosis; 30% with human brain extract and 25% with cerebrospinal fluid. Statistical analyses of the percentage lymphoblastic transformation of lymphocytes of multiple sclerosis patients in response to human brain extract, cerebrospinal fluid, and human myelin basic protein showed no significant difference in their antigenic potency.

Discussion

The in-vitro blastogenic transformation of multiple sclerosis patients in the presence of brain related antigens may be an in-vitro manifestation of delayed hypersensitivity to these antigens since lymphocyte transformation has been correlated with delayed hypersensitivity (Oppenheim, 1968). On the other hand, it is also accepted that lymphoblastogenic transformation in vitro is associated with immunoglobulin production. Since the small peripheral blood lymphocyte is immunologically committed for long periods, it is not striking that we found, as did Fowler *et al.* (1966), that there was no difference in the degree of transformation of multiple sclerosis lymphocytes in relation to the stage of disease activity.

Transformation of sensitive lymphocytes in vitro in the presence of specific antigen is associated with the production of immunoglobulins by these cells (Bartfeld and Juliar, 1964). Studies were not done to demonstrate immunoglobulins present in the cells transformed in response to brain antigens.

The antigenic influence of cerebrospinal fluid may be due to the presence of small amounts of myelin of unique potency. Herndon (1969) observed myelin fragments on electron microscopy of the ultracentrifuged sediment of cerebrospinal fluid from a patient with multifocal leucoencephalopathy.

Multiple sclerosis lymphocyte transformation in vitro in the presence of brain antigens, while pointing to a demyelinating disorder, is not recommended as a specific diagnostic test for multiple sclerosis since Behan *et al.* (1968) found lymphocyte transformation in response to human myelin basic protein in a case of acute haemorrhagic encephalitis and in a patient with postinfectious encephalomyelitis. Greater numbers of patients having various demyelinating diseases should be studied for statistical analysis. The use of lymphocyte transformation tests as an assay of activity in multiple sclerosis cannot be recommended, as we did not find any correlation of the degree of activity and the percentage of transformation.

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