Lymphocyte transformation induced *in vitro* by PHA and PPD in multiple sclerosis

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Altered morphological response of lymphocytes in the presence of antigenic stimulus *in vitro* may constitute an interesting clue to the knowledge of immunological mechanisms in multiple sclerosis (MS). It may serve as a diagnostic test and help in the evaluation of the clinical effect of anti-inflammatory and immunosuppressive agents (Hughes, Caspary, and Field, 1968; Saunders, Knowles, and Field (1969); Cendrowski, 1970). Koulischer and Stenuit (1968) found that lymphocytes from MS patients stimulated by phytohaemagglutinin (PHA) *in vitro* did show reduced transformation during the acute stage of the disease. They thought it resulted from abundant cellular synthesis of G and M immunoglobulins by activated lymphocytes and lowered ability to undergo transformation. Jensen (1968) stated that early transformation after three days' culture was less inhibited than five days' incubation with PHA. Unstimulated lymphocyte transformation from MS patients was normal in heterologous serum from healthy donors, but still reduced in autologous serum, according to Knowles, Hughes, Caspary, and Field (1968).

The present paper reports an attempt to evaluate the degree of lymphocyte transformation in multiple sclerosis, cultured mainly in autologous serum, when dealing with the antigenic stimulus, and the effect of the stage of the disease and immunosuppressive treatment.

CLINICAL MATERIAL AND METHODS

The stimulated and control (unstimulated) cultures of lymphocytes prepared from peripheral blood were carried out in 15 MS patients, in whom the diagnosis was made after complete clinical examination. There were nine women and six men aged from 26 to 56 years, who were fully ambulant with or without the aid of sticks. Emaciated, bed-ridden patients, as well as patients with urinary infections or active tuberculosis were excluded from the study. Nineteen stimulated cultures in autologous and three in heterologous serum from healthy donors were analysed. Two of them were ex-

cluded from analysis because of intensive damage of lymphocytes possibly arising from massive treatment by cyclophosphamide or hydantoin derivatives. The peripheral blood cells were cultured according to the method of Jaroszewicz, Moskalewska, Langner, Piotrowski, and Zych (1968). Phytohaemagglutinin M (PHA-M) (Difco, U.S.A.) and PPD (tuberculin RT23) (Warsaw Manufacture) were used as antigens. In the cultures with PHA and PPD the percentage of transformed cells after 96 hours of incubation was determined using light microscopy. In each culture 1,000 mononuclear cells were examined from a total count. From three patients, lymphocytes in autologous serum were centrifuged (at 450 g for 10 minutes at 18°C), washed three times with the serum of healthy donors, and then cultured in normal serum.

RESULTS

From 11 patients, cultures stimulated by PHA in autologous serum were incubated before immunosuppressive treatment. The mean percentage of transformed cells was found to be 74.7 ± 15.9 . In 20 healthy controls the percentage of cells undergoing transformation and blast-like cells was similar and ranged from 65 to 90% (mean 79%). No difference between these two groups was noted ($t = 0.88$; $0.4 > P > 0.3$). Three patients before immunosuppressive treatment had either acute relapses or showed a slowly progressive course; the proportion of transformed cells was lowered in these subjects to $57.8 \pm 23.5\%$. The difference between them and healthy controls tended to reach statistical significance, which could emerge in larger groups of patients ($t = 1.55$; $0.3 > P > 0.2$). These results are presented in Table.

Over a period of immunosuppressive treatment, cultures stimulated by PHA were carried out in six patients. Four of them received intravenously 200 mg cyclophosphamide per day, one patient was given 6 to 8 mg chlorambucil daily and another was on corticotrophin treatment (75 mg per day). In nearly all patients inhibition of lymphocyte

TABLE

LYMPHOCYTE TRANSFORMATION INDUCED BY PHA *in vitro* IN 15 PATIENTS WITH MULTIPLE SCLEROSIS AND 20 HEALTHY DONORS

	Autologous serum				Heterologous serum
	20 healthy donors	MS patients (11)	MS patients treated by immunosuppressive agents (6)	MS patients with exacerbations or slowly progressive course (3)	MS patients (3)
Percentage of transformed cells	79	74.7 ± 15.9	65.3 ± 19.3	57.8 ± 23.5	38

transformation emerged in ranges from 41 to 87% (mean 65.3 ± 19.3%). The difference compared with healthy controls would indicate a statistically significant level if a larger number of patients were investigated ($t = 1.73$; $P = 0.16$).

One MS patient demonstrated a fairly rare phenomenon. Before immunosuppressive treatment, she had a very reduced number of transformed cells due to PHA stimulation (32.5%). After cyclophosphamide 'cure', the transformation became quite normal (87%). This indicates that the serum cytotoxicity was suppressed by the treatment and lymphocytes recovered their immunological capacity to undergo 'blast' transformation. This notion was supported by slight clinical improvement in the patient's condition (Cendrowski, 1969).

Since the cytotoxic factor from autologous serum may inhibit the response to PHA, lymphocytes from three MS patients were incubated in normal serum and this gave unexpectedly low values from 14 to 61%. It is noteworthy that lymphocytes taken from patients with lymphosarcoma regained normal response to PHA stimulation when the cells were incubated in the serum of healthy donors (Langner, Proniewska, Moskalewska, Gliński, and Polanski, 1969).

In 12 patients 15 cultures stimulated by PPD were set up and analysed. The mean percentage of transformed cells was found to be 0.38 ± 0.77% and did not differ from that observed in controls (0.5%), ($t = 0.6$; $0.6 > P > 0.5$). The response to PPD antigen was positive in two patients and reached a level of 2.0 to 2.5%. This effect occurred in patients who received immunosuppressive agents (cyclophosphamide or ACTH).

DISCUSSION

The lymphocyte transformation stimulated by PHA or PPD *in vitro* showed some deviations in three patients: one had deep inhibition after PHA stimulation, two demonstrated a positive response

to PPD antigen. These findings are fully consistent with that of Brody, Harlem, Kurtzke, and White (1968), but are rather contrary to that of Jensen (1968) and Koulischer and Stenuit (1968).

Lymphocytes of MS patients prepared from peripheral blood did not undergo transformation *in vitro* after incubation with encephalitogenic polypeptide (Behan, Geschwind, Lamarche, Lisak, and Kies, 1968), IgM globulin (Frick and Stickl, 1968) or CSF proteins from MS patients (Brody *et al.*, 1968; Jensen, 1968).

In multiple sclerosis, probably during fresh exacerbations, the response of lymphocytes due to PHA may be impaired. Slight reduction of the number of transformed cells in our three patients argues in favour of this supposition. There is no convincing evidence that lymphocytes from MS patients show immunological deficiency, a deficit of a certain type of RNA, or that they may harbour 'slow virus'. Impaired response to PHA may arise rather from cytotoxic damage of lymphoblasts and blast-like cells by a MS serum factor. The transfer of lymphocytes from the serum of three patients and incubation with PHA in normal serum marked inhibition of transformation. Although this unexpected response is hardly compatible with that of Knowles *et al.* (1968), it seems still possible that PHA could not induce transformation of activated lymphocytes *in vivo*. Lymphocytes, probably activated by brain antigens, synthesize in multiple sclerosis IgG globulin, which may exert a cytotoxic effect.

Kasakura and Lowenstein (1967) discovered that the supernatant of lymphocyte cultures stimulated by PHA or PPD may not only cause 'blast' transformation, but even kill 'blast' cells. Thus certain 'blast' cells might become target cells rather than effectors in delayed type of allergy.

ACTH, cyclophosphamide, and chlorambucil inhibited the transformation, but probably did not suppress the cytotoxicity uniformly. Immunosuppressive treatment did not completely decrease

the immunological response in multiple sclerosis, since a small percentage of lymphocytes underwent morphological changes after PPD stimulation.

In summary, it should be pointed out that slight inhibition of lymphocyte transformation to PHA, when cultured in autologous serum, may arise from the influence of a serum cytotoxic factor. There is also a suggestion that lymphocytes activated by brain antigens *in vivo* do not respond well to PHA stimulation in normal serum.

SUMMARY

Lymphocytes from three multiple sclerosis patients with exacerbations or a slowly progressive course among 11 studied, and from six patients treated by immunosuppressive agents showed slightly reduced transformation by PHA in culture containing autologous serum. When cultured in heterologous serum from healthy donors they also demonstrated inhibited transformation. These findings were possibly related to a MS-serum factor and poor responsiveness to PHA *in vitro* of activated lymphocytes *in vivo*.

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