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Labile Inhibitor of Lymphocyte Transformation in Plasma from a Patient with Subacute Sclerosing Panencephalitis

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A thermolabile inhibitory factor of mitogen-induced lymphocyte transformation was observed in the fresh plasma of one patient in stage 3 of subacute sclerosing panencephalitis (SSPE) and not in three other patients in various stages of the disease. Thus, its relevance to SSPE is not clear. The plasma factor was removed by adsorption onto human and animal cells and polystyrene beads, and it was found in the 7*S* globulin fraction of the effluent from gel permeation chromatography.

Immunological abnormalities have been postulated to be responsible for (2) or associated with (4) subacute sclerosing panencephalitis (SSPE), a chronic fatal measles encephalitis of children and young adults (5, 7). In the present study the leukocytes of four SSPE patients were screened for deficiencies in lymphocyte transformation, and their plasmas were tested for the presence of factors inhibitory to lymphocyte transformation. One patient in stage 3 of SSPE (6) was found to have profound deficiencies in cellular immunity measured by both in vivo and in vitro techniques. A labile factor was found in her plasma which suppressed mitogen-induced deoxyribonucleic acid (DNA) synthesis of both autologous and homologous leukocytes.

MATERIALS AND METHODS

Selection of patients. The diagnoses of SSPE were made clinically and supported by the presence of high measles antibody titers in both the serum and cerebrospinal fluid of the four patients. Plasma, serum, and leukocytes were obtained from these patients and from eight normal volunteers on several occasions.

Preparation and processing of leukocyte cultures. A modification of the method described by Blaese, et al. (1) was used. Leukocytes (1.5×10^6) were added to each vial and suspended in 1 ml of media RPMI 1640 containing penicillin (75 units/ml), streptomycin (50 μg/ml), glutamine (300 mg/ml), and 10% plasma or serum. Stimulants included phytohemagglutinin (PHA; Difco), pokeweed mitogen (PWM; Gibco), *Candida albicans* in 50% glycerin (Holister-Stier), and Streptolysin-O (SLO; Difco).

The PHA- and PWM-stimulated cultures were incubated for 3 days, and the other cultures were incubated for 7 days. Six hours before termination of each culture, 1 μCi of tritiated thymidine (specific activity, -6.0 Ci/mol; Schwartz BioResearch, Inc.) was added. The results were expressed as the average of duplicate or triplicate cultures in counts per minute.

Serum and plasma manipulation. Samples of plasma were frozen at -20 C, placed in a water bath at 56 C for 30 min, or exposed to ultraviolet (UV) light produced by two germicidal bulbs at 20-cm distance for 30 s. This method of UV irradiation inactivated 10^6 plaque-forming units of measles virus in other experiments.

Protein fractionation was performed on a column (100 cm) of Bio-Gel A (0.5 M, 200 to 400 mesh) by using fresh serum or plasma. Samples from the column were pooled into seven fractions corresponding to those areas exhibiting distinctive patterns of optical density, and each fraction was added to PHA-stimulated leukocyte cultures to measure its relative inhibitory capacities.

Portions of fresh plasma were adsorbed with ficoll-hypaque-purified human white and red blood cells, purified chicken and guinea pig spleen mononuclear cells, and suspensions of HeLa cells or polystyrene beads to attempt to remove the inhibitory factor. The effect of 0.1 ml of the adsorbed plasma was assayed in PHA-stimulated leukocyte cultures.

RESULTS

The leukocytes of three SSPE patients (A, B, and C) responded normally in vitro to a variety of nonspecific mitogens such as PHA and PWM and specific stimulants such as *Candida* and

SLO. No inhibitory factors were found in their plasma.

Patient D, however, had profound deficiencies in delayed hypersensitivity and lymphocyte responsiveness. Skin tests with *Candida*, SLO, histoplasmin, coccidiodin, purified protein derivative, diphtheria, and tetanus antigens were repeatedly negative. Initial in vitro studies revealed that the lymphocyte proliferative response of patient D to PHA was inhibited when 10% autologous plasma was present in the leukocyte cultures. Greater response to PHA was observed after removing her plasma and culturing the leukocytes in 10% normal homologous plasma (Table 1).

The remainder of this study deals with an attempt to more specifically characterize the nature of the plasma inhibitory factor found in patient D, and data presented in the tables pertain only to studies on her leukocytes and plasma performed on a number of occasions over a 3-month period.

Specificity of the plasma inhibitor. Fresh plasma from this patient markedly suppressed the mitogen-induced proliferative responses of both autologous and homologous leukocytes (Table 1), but this inhibitory effect could be overcome by using higher concentrations of mitogen or lower concentrations of plasma. Similar results were achieved with PWM. The leukocytes responded normally in vitro to the specific antigenic stimulants (*Candida* and SLO) in either autologous or homologous plasmas, despite the absence of in vivo response as measured by skin tests.

TABLE 1. *Inhibitory effect of SSPE plasma from patient D on phytohemagglutinin-stimulated leukocytes*[a]

Plasma or serum source	Type of plasma or serum	No. of leukocytes[b]	
		SSPE patient	Human volunteer
SSPE patient	Fresh plasma	7.8	52.4
	UV plasma	9.3	99.8
	Frozen plasma (−20 C)	89.7	211.5
	Heated plasma (56 C)	115.1	322.6
	Fresh serum	129.5	175.4
Human volunteer	Fresh plasma	95.6	221.3
Fetal calf	Heated serum (56 C)	118.2	330.8

[a] Phytohemagglutinin concentration was 5 μg/ml of culture media.

[b] Mean counts per minute $\times 10^3$ of triplicate cultures incubated for 3 days and pulsed for 6 h with 1 μCi of tritiated thymidine before termination. Unstimulated control cultures incorporated less than 900 CPM in these experiments.

Lability of the plasma inhibitor. Heating at 56 C for 30 min or freezing at −20 C inactivated the inhibitory factor, but UV treatment did not (Table 1). The inhibitory effect was partially lost when the plasma was incubated at room temperature for 24 h. Addition of fresh complement in the form of fresh human plasma did not restore the inhibitory capacity of the inactivated plasma. The inhibitory factor was also removed by clotting, because the patient's serum did not manifest any inhibitory effect (Table 1).

Agarose column fractionation of the patient's plasma and serum. The major portion of the inhibitory effect of SSPE plasma was concentrated in fraction 3, which contains globular protein (7*S* globulins) with a molecular weight of approximately 150,000 (Table 2). A relatively weaker inhibitory effect was detected in fraction 2. Fraction 6, the column wash, which contained no optical density units at 280 nm, produced a moderate degree of nonspecific inhibition when compared with the PHA control value. Neither SSPE serum from patient D nor plasma or sera from healthy donors manifested an inhibitory effect in fraction 3.

Fresh plasma from patient D did not contain cytotoxic antibodies to human leukocytes as determined by the microcytoxicity assay of Terasaki (kindly performed by R. Yankee, National Cancer Institute).

Adsorbtion of the plasma inhibitor. Ficoll-hypaque-purified human and guinea pig lymphocytes and also red blood cells and polystyrene beads effectively removed the inhibitory factor from the patient's plasma. Goat anti-immunoglobulin with a broad specificity also completely removed the inhibitory factor.

DISCUSSION

An inhibitor of lymphocyte transformation was found in the fresh plasma of one of four SSPE patients. Since leukocytes of this patient responded to mitogen stimulation within the normal range in the presence of normal plasma, there was no apparent intrinsic defect in lymphocyte proliferation. Moreover, the leukocytes

TABLE 2. *Relative depressive effect of agarose column fractions on phytohemagglutinin (PHA)-stimulated normal leukocytes*

Serum or plasma source	Mol wt	SSPE plasma (patient D)[a]	SSPE serum (patient D)	Normal plasma
Column fraction[b]				
1	about 1.5×10^6	42.1[b]	90.5	61.4
2		25.1	57.5	76.7
3	1.6×10^5 (7*S* globulins)	0.26	61.2	66.4
4	6.8×10^4	53.7	61.7	55.6
5	1.3×10^4	46.7	76.2	52.5
6[c]		40.6	69.8	53.0
PHA-stimulated control		123.1		
Unstimulated cell control		0.26		

[a] Mean counts per minute $\times 10^3$ of duplicate cultures.

[b] Samples (0.1 mm) of each fraction added to PHA (5 μg/mm)-stimulated normal leukocytes in the presence of 10% plasma.

[c] Column run-off.

had a proliferative response to specific antigens in vitro in both autologous and homologous plasma, despite the observed negative skin tests to similar antigen preparations of *Candida* and SLO. Because we do not know whether the inhibitory factor was present before this patient developed SSPE and because the factor was not detected in three other patients who were in different stages of disease, the biological significance of this factor remains unclear.

A number of conditions previously associated with plasma inhibitory substances did not pertain in this case. The patient did not manifest signs of renal or hepatic failure or of a malignant disease (8, 9) and she had not received immunosuppressive drugs. UV irradiation of her plasma did not eliminate the inhibitory effect. The dose used would have inactivated any live measles virus which could have inhibited leukocyte DNA synthesis (10).

Inhibitory factors have been detected in serum and plasma from patients with a wide range of disorders, as recently reviewed by R. Gatti (3), but none have been described which have the physical properties of the inhibitory factor detected in patient D.

Because of its extreme lability, the inhibitory factor described can be easily missed if fresh plasma is not evaluated soon after it is drawn.

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