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 7S 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 × 10⁶) 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 10⁴ 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
cultures

CPM in Human vol-

SSPE patient
cies in nature
of No histoplasmin,
mitogen-induced
the responsiveness.
Skin tests with Candida, SLO,
histoplasmin, coccidiolin, purified protein der-
ivative, diphtheria, and tetanus antigens were
repeatedly negative. Initial in vitro studies
revealed that the lymphocyte proliferative re-
sponse of patient D to PHA was inhibited when
10% autologous plasma was present in the
lymphocyte cultures. Greater response to PHA
was observed after removing her plasma and
culturing the leukocytes in 10% normal homolo-
gous plasma (Table 1).

The remainder of this study deals with an
try 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 plas-
mas, despite the absence of in vivo response as
measured by skin tests.

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 comple-
ment in the form of fresh human plasma did not
restore the inhibitory capacity of the inactiva-
ted 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 pa-
tient's plasma and serum. The major portion
of the inhibitory effect of SSPE plasma was
concentrated in fraction 3, which contains globu-
lar protein (7S 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 con-
control value. Neither SSPE serum from patient D
nor plasma or sera from healthy donors mani-
fested an inhibitory effect in fraction 3.

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

Adsorption of the plasma inhibitor. Ficoll-
hypaque-purified human and guinea pig lym-
phocytes and also red blood cells and polysy-
rene 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 lym-
phocyte proliferation. Moreover, the leukocytes

Table 1. Inhibitory effect of SSPE plasma from
patient D on phytohemagglutinin-stimulated
leukocytes

<table>
<thead>
<tr>
<th>Plasma or serum source</th>
<th>Type of plasma or serum</th>
<th>No. of leukocytes*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SSPE patient</td>
</tr>
<tr>
<td>SSPE patient</td>
<td>Fresh plasma</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>UV plasma</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>Frozen plasma (-20°C)</td>
<td>89.7</td>
</tr>
<tr>
<td></td>
<td>Heated plasma (56°C)</td>
<td>115.1</td>
</tr>
<tr>
<td></td>
<td>Fresh serum</td>
<td>129.5</td>
</tr>
<tr>
<td>Human volunteer</td>
<td>Fresh plasma</td>
<td>95.6</td>
</tr>
<tr>
<td>Fetal calf</td>
<td>Heated serum (56°C)</td>
<td>118.2</td>
</tr>
</tbody>
</table>

* Phytohemagglutinin concentration was 5 µg/ml of culture media.

* Mean counts per minute x 10⁴ of triplicate cultures incubated for 3 days and pulsed for 6 h with 1
µCi of tritiated thymidine before termination. Un-
stimulated control cultures incorporated less than 900
CPM in these experiments.
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.

ACKNOWLEDGMENTS

We acknowledge the cooperation of John L. Sever, Chief of Infectious Disease Branch, National Institute of Neurological Diseases and Stroke, in providing some of the patients' blood samples, and also the excellent technical assistance of S. Dougherty.

LITERATURE CITED


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

<table>
<thead>
<tr>
<th>Serum or plasma source</th>
<th>Mol wt</th>
<th>SSPE plasma (patient D)</th>
<th>SSPE serum (patient D)</th>
<th>Normal plasma</th>
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</thead>
<tbody>
<tr>
<td>Column fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>about 1.5 x 10^4</td>
<td>42.1^b</td>
<td>90.5</td>
<td>61.4</td>
</tr>
<tr>
<td>2</td>
<td>1.6 x 10^4 (7S globulins)</td>
<td>25.1</td>
<td>57.5</td>
<td>76.7</td>
</tr>
<tr>
<td>3</td>
<td>6.8 x 10^4</td>
<td>0.26</td>
<td>61.2</td>
<td>66.4</td>
</tr>
<tr>
<td>4</td>
<td>1.3 x 10^4</td>
<td>53.7</td>
<td>55.6</td>
<td>61.7</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>69.8</td>
<td>52.5</td>
<td>61.7</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>53.0</td>
<td>69.8</td>
<td>66.4</td>
</tr>
<tr>
<td>PHA-stimulated control</td>
<td></td>
<td>123.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstimulated cell control</td>
<td></td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Mean counts per minute x 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.