A PRELIMINARY REPORT ON THE NEUTRALIZATION OF
THE LYMPHOCYTOSIS-PROMOTING FACTOR (LPF)
WITH ANTI-LPF SERUM

Ishida (1968) described neutralization of the mouse body weight-decreasing
toxicity of the lymphocytosis-promoting factor (LPF) with an anti-LPF rabbit
serum. The present preliminary report describes some anti-LPF properties of
the antiserum, which will give some explanation as to the possible mechanism
of the development of lymphocytosis by LPF.

A preparation of LPF, 78E, and an anti-LPF rabbit serum, APr1-1 (Ishida,
1968), were used in the present experiments. The antiserum neutralized the body
weight-decreasing toxicity of LPF (Ishida, 1968) and inhibited the increase in
peripheral leukocytes (Fig. 1) almost completely when LPF was mixed with the
antiserum in vitro before inoculation.

The counting of total peripheral leukocytes can be employed as an alternative
method for the quantitation of the LPF-induced lymphocytosis, because the increase
in peripheral leukocytes by LPF inoculation is essentially due to that in small
lymphocytes (Ishida et al., 1968; Kurokawa, et al., 1970). Each group of 10 mice
was given APr1-1 intraperitoneally in the amount of 0.06 ml 1, 2, 3 or 4 days
after the intraperitoneal inoculation of 1.3 D0D4* of 78E and examined daily for
the peripheral leukocytes and body weight change. The bleeding for counting
leukocytes was made every day from 1 day after the antiserum inoculation alter-
nately from one half and the other half of the mice of each group. Another
group of 15 mice was inoculated with LPF alone to see the activity of the LPF
preparation. Three subgroups of an equal number were bled alternatively.
Another group of 5 mice given no LPF was bled for counting normal peripheral
leukocytes. Leukocyte countings were made microscopically with a Thoma-Zeiss'
hemocytometer. The results are presented in Fig. 2.

The anti-LPF serum neutralized both the lymphocytosis-promoting and the
body weight-decreasing activities of LPF even when given 4 days after the LPF
inoculation.

It is considered to be a common feature of antitoxic immunity that, once the
cells susceptible to the toxin have been attacked, the antitoxin is relatively in-
effective (Wilson and Miles, 1964). Though LPF has shown every property
common to exotoxins (Iwasa et al., 1968; Ishida, 1968), the recovery from the
LPF-induced lymphocytosis and the body weight decrease was independent of the
intervals between the LPF and the anti-LPF serum inoculation, with the exception
of two groups in which the serum was given 1 or 2 days after LPF and the body

* The dose of LPF at which the mean body weight of mice would neither increase nor
decrease from the 2nd day to the 4th day. This was proposed as an absolute unit of the
Fig. 1. Neutralization of the lymphocytosis-promoting activity with the anti-LPF serum.

A mixture of 1.5 $D_0D_4$ of 78E and 0.06 ml of APr1-1 was left standing at room temperature for 30 min and injected intraperitoneally into 10 mice. Half of the mice was bled from the tail vein on days 0, 2 and 4, and the other half on days 1, 3 and 5. For a control 0.06 ml of serum from a normal rabbit was used.

One-hundredth ml of the blood was added to 10 ml of a buffered saline (pH 7.2) and the mixture was vigorously shaken, and added with 0.3 ml of a 1% saponin solution. Counting of leukocytes was made electronically with a Coulter Counter, model B (Coulter Electronics Inc., Hialeah, Fla.).

weight decreased once on day 3 and then began to increase. This phenomenon of the body weight recovery will be discussed in a separate paper.

In the studies on the mechanism of the development of lymphocytosis in rats given LPF, Iwasa et al. (1970) obtained some evidences showing direct adsorption of LPF onto lymphocytes. These and the present findings may constitute a hypothesis that LPF attaches loosely and reversibly upon small lymphocytes to render them unable to migrate from the blood stream to the lymph nodes without giving any profound damage to the cells. More direct evidences, however, will be necessary to justify this hypothesis.

REFERENCES


Fig. 2. Neutralization of LPF with the anti-LPF serum inoculated at various times after LPF injection.

a. Change in peripheral leukocyte population after the anti-LPF serum inoculation.

b. Change in body weight after the anti-LPF serum inoculation.

Though the measurement of body weight was made every day on every mouse, the counting of peripheral leukocytes was made from the day next to the serum inoculation on two subgroups alternately as stated in the text.

Group 1: Control (LPF alone).
Group 2, 3, 4, and 5 were inoculated with anti-LPF serum 1, 2, 3, and 4 days, respectively, after LPF.
Group 6: Control (no LPF).

* The difference in g. between the body weight on the day of material inoculation and that on the ith day (Kurokawa et al., 1962; Ishida, 1968).

Summary of variance analysis for the data of Fig. 2

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Probability level (p) at which the difference was significant**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total leukocytes</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

** Significance tests were made for the difference in the total number of peripheral leukocytes or the body weight difference between the control group (group 1) and each of the LPF groups (groups 2, 3, 4 and 5).
pertussis vaccine using mice. II. Some considerations on the level of toxicity passing

of toxicity of pertussis vaccine. III. Quantitative determination of the lymphocytosis-
promoting factor by leukocyte counting. (To be submitted to this journal).


Department of General Biologics Control,
National Institute of Health,
Shinagawa-ku, Tokyo 141, Japan

Received: November 24th, 1969

SETSUJI ISHIDA
SADAO ASAKAWA
MASAMI KUROKAWA

石田慎作・朝川貞雄・黒川正身（国立予防衛生研究所一般検定部）