Augmentation of the Antibody Response by Lipoic Acid in Mice
II. Restoration of the Antibody Response in Immunosuppressed Mice

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Abstract—Lipoic acid (Lip) was examined for its effect on the in vivo antibody response in mice. It was found that Lip significantly restored the suppressed antibody response to sheep erythrocytes (SRBC) in cyclophosphamide-injected mice when it was orally administered at 25 mg/kg twice a day for 4 consecutive days after immunization. On the other hand, Lip-administration did not affect the antibody response in normal mice immunized with SRBC. Normal or cyclophosphamide-treated mice were primed with horse erythrocytes (HRBC) and were given either saline or Lip for 3 consecutive days. HRBC-specific helper T cell activity of their spleen cells were compared by coculturing these cells with trinitrophenyl (TNP)-keyhole limpet hemocyanin-primed spleen cells in the presence of TNP-HRBC as the antigen. A significant restoration of the suppressed helper T cell activity was observed in the Lip-administered group. However, the helper T cell activity was not affected significantly by Lip-administration in normal mice. Lip could also partially restore the helper T cell activities that were suppressed by the treatment with hydrocortisone or X-ray irradiation.

It has been reported that various thiol and disulfide compounds are able to augment the in vitro antibody response (1–5). In the preceding paper (6), we demonstrated that lipoic acid (Lip), a naturally occurring disulfide compound showed augmenting effects on in vitro antibody responses in murine lymphocytes. It was suggested that the mode of action of Lip was to stimulate antigen-specific helper T cells (6). If Lip would show an augmenting effect on the antibody response in vivo as well as in vitro, this compound might be expected to be an immunostimulant for therapeutic use. Lip has been reported to be effective for the therapy of liver damages and heavy metal poisoning (7). In the present paper, we report that Lip was able to restore the antibody response in immunosuppressed mice.

Materials and Methods

Materials: Female BALB/c mice (6 weeks of age) were purchased from Shizuoka Agricultural Cooperation of Experimental Animals (Shizuoka) and were used during 10–15 weeks of age. Hydrocortisone was obtained from Sigma Chemical Company. The cyclophosphamide (CY) used was Endoxan® (Shionogi). Other materials used in this paper were purchased or prepared as described in the preceding paper (6).

Antibody response in vivo: A group of 4 mice was immunized with the intravenous injection of \(1 \times 10^8\) sheep erythrocytes (SRBC). Four days later, mice were sacrificed, and, their spleen cells were examined for the number of anti-SRBC plaque-forming cells (PFC) according to Jerne's hemolytic plaque assay (8). In some groups, 50 mg/kg CY was injected intraperitoneally 1 day and 2 days before immunization. Lip was orally adminis-
tered at 25 mg/kg twice a day for 4 con-
secutive days starting from the day of im-
umunization. The solution of Lip was pre-
pared by dissolving 50 mg of Lip (free acid) in 10 ml of saline containing an equivalent amount of NaOH.

Induction of antigen-specific helper T
cells in vivo and the assay of their activities
in vitro: Immunosuppressed mice used in
these experiments were prepared by the
following three procedures: A) CY was
administered intraperitoneally at 80 mg/kg
day -2) and at 50 mg/kg (day -1) prior to
immunization. B) Hydrocortisone was in-
jected intraperitoneally at 125 mg/kg 2 days
before immunization. C) Mice were irradiated
with 150 R X-rays 3 days before priming with
the antigen.

Normal or immunosuppressed mice (N=3)
were primed with the intravenous injection
of 2X10^6 horse erythrocytes (HRBC). Three
days later, HRBC-specific helper T cell
activities of their spleen cells were estimated
by the following two methods: I) Spleen cells
from HRBC-primed mice (6X10^6) were
cultured in triplicate with 2X10^6 SRBC or
5X10^5 trinitrophenyl (TNP)-HRBC in 2 ml
of RPMI-1640 medium containing 10% fetal
calf serum at 37°C for 5 days under 5% CO2
and 95% air. Anti-SRBC or anti-TNP IgM
PFC were enumerated by using SRBC or
TNP-SRBC, respectively. TNP-HRBC and
TNP-SRBC were prepared as described by
Rittenberg and Pratt (9).

II) The same HRBC-primed cells as above
(5X10^5) were cocultured in triplicate with
6X10^6 TNP-keyhole limpet hemocyanin
(KLH)-primed cells in the presence of TNP-
HRBC (5X10^5) for 5 days as described above.
Both IgM and IgG anti-TNP PFC were
enumerated by using TNP-SRBC as indicator
cells (10). HRBC-specific helper T cell
activity could be assayed in this procedure.

Mice were primed with TNP-KLH as described
previously (6, 10). In order to investigate
the effect of Lip on helper T cell activity, Lip
was orally administered to mice at 25 mg/kg
twice a day for 3 days starting from the day
of immunization.

All the data were presented as the mean
number of PFC±standard error and analyzed
with Student's t-test.

Results

Effect of Lip administration on the antibody
response in normal or immunosuppressed
mice: Lip administration did not affect the
antibody response in normal mice that were
immunized intravenously with an optimal
dose of SRBC as illustrated in Fig. 1. The
antibody response was suppressed by 35–
40% in the mice that were injected with 50
mg/kg CY twice before or after the immuni-
zation as indicated in Fig. 2. The suppressed
immune response was found to be signifi-
cantly restored (P<0.01) when mice
were given 25 mg/kg Lip twice a day for 4
consecutive days starting from the day of
immunization (Fig. 2).

Effect of Lip administration on the sup-
pressed helper T cell activity in CY-treated
mice: In the preceding paper (6), we pre-
presented data indicating that Lip would exert
its immunostimulating effects through en-
hancing helper T cell activity by employing
an in vitro culture system. It was demonstrated
in the above section that Lip was able to
restore the suppressed antibody response in
CY-treated mice. Then, we investigated
whether Lip would enhance helper T cell
activity specifically or show nonspecific stimulating effects on all the immunocompetent cells when Lip was administered to mice. Mice (N=3) were immunized intravenously with 2 x 10^6 HRBC. After 3 days, their spleen cells were pooled and cultured with SRBC or TNP-HRBC. Anti-SRBC or anti-TNP PFC were enumerated after 5 days. As shown in Table 1, antibody responses were drastically suppressed in the lymphocytes from mice

![Graph](image)

Fig. 2. Effect of Lip administration on the suppressed antibody response in CY-injected mice. Mice (N=4) were immunized intravenously with 1 x 10^6 SRBC. CY (50 mg/kg) was injected twice intraperitoneally before (Expt. 2) or after (Expt. 1) immunization. Lip was orally administered at 25 mg/kg twice a day according to the schedule indicated in the figure. The control group received saline in place of Lip. CY, Lip is significantly different from CY, saline (P<0.01).

Table 1. Effect of lipoic acid administration on the suppressed antibody response in cyclophosphamide-treated mice

<table>
<thead>
<tr>
<th>Treatment of mice</th>
<th>PFC/culture</th>
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<tbody>
<tr>
<td></td>
<td>SRBC</td>
</tr>
<tr>
<td>Expt. 1</td>
<td></td>
</tr>
<tr>
<td>Normal + saline</td>
<td>4350±150</td>
</tr>
<tr>
<td>Normal + Lip (25 mg/kg x 2/day)</td>
<td>4545±195</td>
</tr>
<tr>
<td>CY + saline</td>
<td>30±15</td>
</tr>
<tr>
<td>CY + Lip (25 mg/kg x 2/day)</td>
<td>75±25</td>
</tr>
</tbody>
</table>

| Expt. 2           |             |             |
| Normal + saline   | 5660±240    | 2170±90     |
| CY + saline       | 60±20       | 60±40       |
| CY + Lip (25 mg/kg x 1/day) | 60±10  | 260±60**   |
| CY + Lip (25 mg/kg x 2/day) | 90±10    | 550±90*    |

* Mice (N=3) were injected intravenously with 2 x 10^6 HRBC. After 3 days, their spleen cells were cultured with SRBC or TNP-HRBC for 5 days as described under Materials and Methods. In some groups, CY was intraperitoneally injected at 80 mg/kg on day -2 and 50 mg/kg on day -1 before immunization. Lip was orally administered at 25 mg/kg once or twice a day according to the schedule indicated below. *P<0.01 and **P<0.05 versus CY+saline.
that were injected with CY twice before immunization. In normal mice, Lip-administration did not affect the immune responses to both SRBC and TNP-HRBC. In contrast, the antibody response to TNP-HRBC was markedly restored in CY-injected mice when mice were administered with 25 mg/kg Lip twice a day for 3 days according to the schedule shown in Table 1. Anti-SRBC response, however, was improved to only a small extent by the same treatment. These results suggest that Lip did not nonspecifically augment the activities of various immunocompetent cells, but it specifically stimulated helper T cells that were primed with HRBC.

As indicated in Expt. 2 of Table 1, Lip could augment the antibody response more effectively when it was administered twice a day.

In order to investigate that HRBC-specific helper T cell activity was really augmented by Lip administration, a small number of HRBC-primed cells \((5 \times 10^3)\) that were the same as those used in Expt. 1 of Table 1 were cocultured with TNP-KLH-primed spleen cells \((6 \times 10^6)\) in the presence of TNP-HRBC as the antigen. As shown in Table 2, a high helper T cell activity was induced in normal HRBC-primed mice. Lip administration did not affect the helper T cell activity in normal mice.
mice. However, the suppressed helper T cell activity in CY-injected mice was restored by the administration of Lip. In another experiment not shown here, we confirmed that helper cell activity of HRBC-primed spleen cells were HRBC-specific and was diminished by the treatment with anti-Thy-1,2 plus complement.

Restoration of helper T cell activity by Lip in various immunosuppressed mice: Lip was examined for its effect on the helper T cell activities that were suppressed by various means other than CY-injection. Helper T cell activity was estimated by coculturing HRBC-primed cells with TNP-KLH-primed cells as indicated in Table 2. In hydrocortisone-injected mice, HRBC-specific T cell activity was lowered to approximately 50% of that in normal mice, but was restored by Lip administration as shown in Table 3. Although the data are not shown here, restoration by Lip of the suppressed antibody response was similarly observed when HRBC-primed lymphocytes were directly cultured with TNP-HRBC by the same procedure as that in Table 1.

In addition, Lip administration was effective for the restoration of the suppressed helper T cell activity in X-ray-irradiated mice. As shown in Table 4, X-ray irradiation resulted in the suppression of helper T cell activity to 1/2–1/3 of that in control mice. When Lip was administered to irradiated mice according to the schedule shown in Table 4, HRBC-specific helper T cell activity was markedly restored. However, the restoration by Lip was not observed in the response to an irrelevant antigen like SRBC when the spleen cells from three mice were directly cultured with SRBC (Data not shown).

**Discussion**

In the present paper, it was shown that Lip was effective in restoring the antibody responses of mice that were suppressed by pretreating the animals with CY, hydrocortisone or X-rays. The number of spleen cells was reduced to 30–50% of the control by these treatments. Lip administration did not significantly restore the decreased cell number, although it could augment the suppressed antibody responses. These results suggest that Lip does not stimulate the proliferation of murine lymphocytes nonspecifically, but would augment the antibody response by enhancing the activity of a specific clone of helper T cells. This will be supported by the data in Tables 2–4 that Lip could augment the antigen-specific helper T cell activities that were suppressed by various means. This was also consistent with our previous data of in vitro experiments that

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**Table 4.** Effect of lipoic acid administration on the suppressed helper T cell activity in X-ray-irradiated mice

<table>
<thead>
<tr>
<th>Helper T cell source (Treatment of mice)</th>
<th>Anti-TNP PFC/culture*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IgM PFC</td>
</tr>
<tr>
<td>None</td>
<td>690±270</td>
</tr>
<tr>
<td>Normal + saline</td>
<td>1670±431</td>
</tr>
<tr>
<td>irradiated a – saline</td>
<td>760±28</td>
</tr>
<tr>
<td>irradiated + Lip b</td>
<td>1200±30*</td>
</tr>
</tbody>
</table>

a Mice (N=3) were irradiated with 150 R X-rays 3 days before immunization with 2×10⁶ HRBC. b Lip was orally administered at 25 mg/kg twice a day for 5 days as indicated below. The control group received saline. c Three days after immunization, HRBC-primed spleen cells (5×10⁶) were cocultured with TNP-KLH-primed cells (6×10⁶) in the presence of 5×10⁶ TNP-HRBC as indicated under Materials and Methods. *P<0.01 and **P<0.05 versus irradiated+saline.
Lip could not stimulate B cells directly, but could augment the activity of helper T cells which were stimulated by the antigen (HRBC) (6).

Lip did not augment or suppress the antibody response when it was administered to normal mice (Fig. 1 and Table 1). On the other hand, Lip could no more enhance the secondary antibody response to SRBC in vitro (Data not shown). These data imply that Lip could not further stimulate the lymphocytes (helper T cell) when the cells were activated to the greatest degree under optimal conditions. This appears to be convenient for the therapeutic use of Lip because the drug would not stimulate the lymphocytes excessively.

Immunosuppressed states are often generated in the treatment of cancer by irradiation, antitumor drugs or surgery and in the treatment of autoimmune diseases by immunosuppressive agents. It is important to improve these conditions of impaired immunity by some therapeutic agent. It has been reported that levamisole was effective in recovering the responsiveness of human lymphocytes to mitogens that had been depressed by surgical intervention or irradiation in cancer patients (11, 12). However, levamisole is known to have an undesirable side effect like causing granulocytopenia (13). Lip has been known to be safely administered to the body for the cure of heavy metal poisoning or liver damages (7). It appears worth testing whether this new pharmacological effect of Lip on the immune response is applicable for clinical use.

References