Interleukin 2 Requirement for the in Vitro Generation of Antitumor Cytotoxicity by Thymocytes from Melphalan-cured MOPC-315 Tumor Bearers

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ABSTRACT

We have previously shown that enhanced antitumor cytotoxicity is generated when thymocytes from melphalan (L-phenylalanine mustard; L-PAM)-treated MOPC-315 tumor bearers, but not thymocytes from normal mice, are added to the immunization culture of syngeneic normal spleen cells and MOPC-315 tumor cells (Bartik et al., Cancer Res., 47: 4848-4855, 1987). Here we show that normal spleen cells produce, upon stimulation with MOPC-315 tumor cells, helper-like factors which are sufficient for thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not for thymocytes from normal mice, to develop antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells. Since one of the helper-like factors produced by in vitro-immunized spleen cells is interleukin 2 (IL-2), we assessed the exogenous IL-2 requirements for the development of anti-MOPC-315 cytotoxicity in thymocytes from L-PAM-treated MOPC-315 tumor bearers, relative to thymocytes from normal mice. Thymocytes from L-PAM-treated MOPC-315 tumor bearers were found to require a 10-fold lower concentration of recombinant IL-2 (rIL-2) than thymocytes from normal mice in order to develop antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells. The concentration of rIL-2 required for the development of anti-MOPC-315 cytotoxicity by thymocytes from L-PAM-treated MOPC-315 tumor bearers was also 10-fold lower than the concentration of rIL-2 required by thymocytes from untreated MOPC-315 tumor bearers or thymocytes from L-PAM-treated normal mice. In addition, at any concentration of rIL-2 employed, thymocytes from L-PAM-treated MOPC-315 tumor bearers developed a higher level of anti-MOPC-315 cytotoxicity than did thymocytes from normal mice, L-PAM-treated normal mice, or untreated MOPC-315 tumor bearers. The enhanced antitumor cytotoxicity exhibited by thymocytes from L-PAM-treated MOPC-315 tumor bearers, following in vitro stimulation with MOPC-315 tumor cells plus rIL-2, was evident not only against MOPC-315 tumor cells but also against other syngeneic plasmacytomas but not an allogeneic thymoma. In addition, thymocytes from L-PAM-treated MOPC-315 tumor bearers required less rIL-2 than thymocytes from normal mice to develop antitumor cytotoxicity in response to stimulation with MOPC-315-associated antigens but not in response to stimulation with an allogeneic genetically unrelated thymoma (ELA). Thus, L-PAM treatment of MOPC-315 tumor bearers, but not of normal mice, leads to a substantial reduction in the concentration of exogenous IL-2 required by their thymocytes in order to develop an antitumor cytotoxic response against plasmacytoma-associated antigens.

INTRODUCTION

The immunosuppressive potential of anticancer drugs has been recognized for some time (1). However, it is now clear that the effect of anticancer drugs on the immune system is not uniformly suppressive. In fact, many investigators have shown that under defined conditions anticancer drugs can actually enhance a variety of immune responses (2–13). Specifically, it was shown that exposure of lymphoid cells to anticancer drugs prior to their antigenic or mitogenic stimulation leads to the appearance of a greatly enhanced immune response as a consequence of drug-mediated impairment of suppressor cell activity. In these studies, the anticancer drugs were shown to reduce the suppressor cell pool either through selective toxicity for precursors of suppressor T-cells (4, 7, 11) or as a result of a slower recovery of the suppressor cell activity under conditions in which the drug was toxic for precursors of suppressor T-cells as well as for other subsets of T-cells (9). Another mechanism by which anticancer drugs were shown to enhance immune responses is through stimulation of helper T-cells to produce elevated levels of IL-2 which in turn facilitates the generation of cytotoxic T-lymphocytes (14, 15).

The documentation that pretreatment with anticancer drugs can potentiate the generation of immune responses has stimulated several attempts to exploit anticancer drugs to enhance the development of antitumor immunity in tumor bearers (12, 16–21). Indeed, anticancer drugs were found to be successful in several animal tumor models in shifting the balance from immunosuppression to potent antitumor immunity (6, 10, 12, 17). Moreover, the anticancer drug cyclophosphamide was found to enhance the ability of patients with advanced melanoma to develop a delayed-type hypersensitivity response to melanoma-associated antigens following immunization with an autologous tumor vaccine (18, 19) and to develop LAK-like cells following administration of IL-2 (21). Both protocols were associated with clinical improvements in some of the patients.

Employing the murine MOPC-315 plasmacytoma we have previously shown that a low dose of cyclophosphamide or L-PAM can bring about the rapid appearance of potent antitumor immunity in mice bearing a large tumor that prior to the chemotherapy did not exhibit concomitant antitumor immunity as evidenced by their inability to reject a tumor challenge (2, 16). Further studies revealed that the immunopotentiating activity of the low dose of L-PAM is evident not only in the intact mice but also in their thymuses (22, 23). Accordingly, L-PAM therapy rendered thymocytes from MOPC-315 tumor bearers capable of bringing about the generation of enhanced antitumor cytotoxicity when added to the immunization culture of normal spleen cells and MOPC-315 tumor cells. At the same time, L-PAM administered to normal mice was unable to render their thymocytes capable of bringing about the generation of enhanced antitumor cytotoxicity when added to the immunization culture of normal spleen cells.

The current study was undertaken to gain some insight into the mechanism through which thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not thymocytes from normal mice, bring about the generation of enhanced antitumor cytotoxicity when added to the immunization culture of normal spleen cells and MOPC-315 tumor cells. Accordingly, we examined the possibility that even though thymocytes from L-PAM-treated MOPC-315 tumor bearers, like thymocytes from normal mice, do not develop an antitumor cytotoxic response when immunized in vitro with MOPC-315 tumor cells in the

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4 The abbreviations used are: IL-2, interleukin 2; L-PAM, L-phenylalanine mustard; rIL-2, recombinant IL-2; LAK, lymphokine-activated killer.
absence of normal spleen cells (22), such thymocytes, in contrast to thymocytes from normal mice, may be able to generate anti-MOPC-315 cytotoxicity when immunized in vitro in the presence of helper-like factors produced by normal spleen cells subjected to in vitro immunization. In addition, since one of the helper-like factors produced by normal spleen cells subjected to in vitro immunization is IL-2 (24), we determined the exogenous IL-2 requirements of thymocytes from L-PAM-treated MOPC-315 tumor bearers relative to that of thymocytes from normal mice in order to develop antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells. As part of this study, we also established the exogenous IL-2 requirements for the generation of antitumor cytotoxicity by thymocytes from untreated MOPC-315 tumors and by thymocytes from L-PAM-treated normal mice. Finally, we compared thymocytes from L-PAM-treated MOPC-315 tumor bearers and thymocytes from normal mice in terms of their exogenous IL-2 requirements for the generation of antitumor cytotoxicity against other plasmacytomas and even a thymoma.

MATERIALS AND METHODS

Tumors. We have employed primarily the MOPC-315 plasmacytoma of BALB/c origin, which is considered a weakly immunogenic tumor (25). The MOPC-315 was maintained in vivo as a s.c. tumor in female BALB/c mice 7 to 10 weeks old (Charles River Breeding Laboratories, Wilmington, MA), as we have previously described (26). In addition to the MOPC-315 plasmacytoma we have employed two other plasmacytomas of BALB/c origin, the MOPC-104E and the RPC-5. While the MOPC-315 cells possess surface IgA-λ antibody with specificity for nitrophenyl groups (27, 28), the MOPC-104E cells possess surface IgM-λ with specificity for dextran, and the RPC-5 cells possess surface IgGn-k with unknown binding specificity (29). Finally, in addition to the plasmacytomas, we have employed the EL4 thymoma of C57BL/6 origin.

Chemotherapy. A fresh stock solution of 10 mg/ml L-PAM was prepared just prior to injection as previously described (2) and was further diluted with Dulbecco’s phosphate buffered saline, pH 7.2 (Grand Island Biological Co., Grand Island, NY), to the desired concentration. A dose of 2.5 mg of L-PAM per kg of body weight (2.5 mg/kg) was immediately administered i.p. to normal mice or mice bearing a s.c. MOPC-315 tumor with a diameter of 20–22 mm resulting from the inoculation of 1 × 10⁶ MOPC-315 tumor cells 10 days earlier. This dose of drug is the lowest dose of L-PAM which is curative for at least 90% of mice bearing a large (≥20 mm) MOPC-315 tumor (2).

Lymphoid Cell Suspensions. Single cell suspensions were prepared from the spleens and thymuses of normal mice. In addition, we prepared single cell suspensions from the thymuses of: (a) normal mice that received an i.p. injection of 2.5 mg/kg L-PAM 12 days earlier, (b) mice bearing a 20–22 mm s.c. MOPC-315 tumor as a result of inoculation with 1 × 10⁶ tumor cells 10 days earlier, and (c) mice that were treated with 2.5 mg/kg L-PAM 10–12 days earlier when they bore a Day 10 MOPC-315 tumor with a diameter of 20–22 mm. Since the dose of L-PAM used leads to the complete rejection of the s.c. tumor within 8 days (2), the thymocyte donors in the L-PAM-treated MOPC-315 tumor bearer group represent mice that completed the rejection of the s.c. tumor 2–4 days prior to the excision of their thymuses. In any individual experiment, the thymuses and the spleens were obtained from at least three mice/group. Single cell suspensions were prepared by mechanical disruption between glass slides as previously described (26), and the viability as determined by trypan blue dye exclusion (>95%) always exceeded 95%.

In Vitro Immunization. When thymocytes were immunized in vitro in the presence of soluble factors produced by normal spleen cells stimulated in vitro with MOPC-315 tumor cells, 10 × 10⁶ thymocytes were placed in a culture plate insert (12 mm in diameter) containing a permeable membrane with 0.45-µm pores (Millipore, Bedford, MA) which in turn was placed in a 6-well Costar plate (Costar, Cambridge, MA) containing 20 × 10⁶ spleen cells. Mitomycin C-treated (50 µg/ml for 30 min) MOPC-315 tumor cells were added to the chamber containing the spleen cells as well as the chamber containing the thymocytes. The ratio of lymphoid cells to tumor cells employed in these studies was 30/1, which is the optimal ratio for the generation of antitumor cytotoxicity by normal spleen cells (26). When thymocytes were immunized in vitro in the presence of rIL-2, 40 × 10⁶ thymocytes were admixed with 1.33 × 10⁶ mitomycin C-treated stimulator tumor cells and human rIL-2 (which was generously provided by Cetus Corporation, Emeryville, CA) in a 30-ml tissue culture flask (Corning Glass Works, Corning, NY). All in vitro immunizations were carried out for 5 days at 37°C in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, 1% nonessential amino acids, 50 units/ml penicillin, 50 µg/ml streptomycin (Grand Island Biological Co.) and 5 × 10⁻⁵ m 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO) (26).

Antitumor Cytotoxicity Assay. Cell-mediated immune lysis was determined as described previously utilizing the 3.5-h ⁵¹Cr-release assay (26). The percentage of specific ⁵¹Cr release was calculated by the following formula:

\[
\% \text{ of specific } ^{51}\text{Cr release} = \left( \frac{T - C}{M - C} \right) \times 100
\]

where T is the percentage release with test lymphocytes, C is the percentage of spontaneous release (which was about 15% with EL4 target cells and 17–25% with MOPC-315, MOPC-104E, or RPC-5 target cells), M is the maximal release obtained by three cycles of freezing and thawing (which ranged between 85–92% for all types of target cells employed). Each experiment was performed three to five times. We have observed (23, 30), as have others (31), that the level of antitumor cytotoxicity obtained with lymphoid cells of individual mice immunized under the same culture conditions may vary substantially from one experiment to another. Still, the pattern of the results remained consistent. The level of antitumor cytotoxicity is presented as the mean of ⁵¹Cr release of triplicate samples ± SE. Variations in the percentage of ⁵¹Cr released between individual samples rarely exceeded 5%.

RESULTS

Ability of Thymocytes from L-PAM-treated MOPC-315 Tumor Bearers to Generate an Antitumor Cytotoxic Response following Stimulation with MOPC-315 Tumor Cells in the Presence of Soluble Factors Produced by Normal Spleen Cells Immunized in Vitro with MOPC-315 Tumor Cells. We have previously shown that although thymocytes from L-PAM-treated MOPC-315 tumor bearers, like thymocytes from normal mice, are unable to generate an antitumor cytotoxic response when immunized in vitro with MOPC-315 tumor cells, thymocytes from L-PAM-treated MOPC-315 tumor bearers, in contrast to thymocytes from normal mice, can bring about the generation of enhanced antitumor cytotoxicity when added to the immunization culture of normal spleen cells and MOPC-315 tumor cells (22, 23). Experiments were performed to determine whether normal spleen cells produce, upon in vitro immunization with MOPC-315 tumor cells, helper-like factors sufficient for the generation of antitumor cytotoxicity by thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not by thymocytes from normal mice. For this purpose, thymocytes from normal mice or from L-PAM-treated MOPC-315 tumor bearers were separated from normal spleen cells via a permeable membrane that allowed the exchange of soluble factors, but not cells, between the spleen cells and the thymocytes. Both the spleen cells and the thymocytes were subjected to stimulation with MOPC-315 tumor cells. As can be seen from Table 1, thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not thymocytes from normal mice, developed a significant level of antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells.
ENHANCEMENT OF THYMIC ANTITUMOR IMMUNE REACTIVITY

Table 1  Level of antitumor cytotoxicity generated by thymocytes from normal mice or L-PAM-treated MOPC-315 tumor bearers when immunized in vitro with MOPC-315 tumor cells in the presence of soluble factors released from normal spleen cells immunized in vitro with MOPC-315 tumor cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Normal mice</th>
<th>L-PAM-treated tumor bearers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7 ± 0.2</td>
<td>22.6 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>4.0 ± 0.9</td>
<td>12.5 ± 5.0</td>
</tr>
<tr>
<td>3</td>
<td>5.3 ± 1.7</td>
<td>13.3 ± 0.4</td>
</tr>
</tbody>
</table>

a Effector-to-target-cell ratio of 100/1.

315 tumor cells in the presence of the soluble factors produced by the normal spleen cells immunized in vitro with MOPC-315 tumor cells. Thus, the soluble factors produced by normal spleen cells immunized in vitro with MOPC-315 tumor cells are sufficient to enable thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not thymocytes from normal mice to develop an antitumor cytotoxic response following stimulation with MOPC-315 tumor cells.

Assessment of the Concentration of Exogenous IL-2 Required for the Generation of Antitumor Cytotoxicity in Thymocytes from Normal Mice or from L-PAM-treated MOPC-315 Tumor Bearers in Response to in Vitro Stimulation with MOPC-315 Tumor Cells. Since one of the helper factors produced by normal spleen cells upon in vitro immunization is IL-2 (24), we evaluated the IL-2 requirements of thymocytes from L-PAM-treated MOPC-315 tumor bearers, relative to thymocytes from normal mice, for the development of anti-MOPC-315 cytotoxicity. For this purpose, thymocytes from L-PAM-treated MOPC-315 tumor bearers or thymocytes from normal mice were cocultured with MOPC-315 tumor cells in the presence of various concentrations of rIL-2 ranging from 0 to 15.0 units/ml (Fig. 1). As expected (22), in the absence of rIL-2, thymocytes from either normal mice or L-PAM-treated MOPC-315 tumor bearers were unable to develop antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells. As the concentration of rIL-2 added to the immunization cultures was increased, a higher level of anti-MOPC-315 cytotoxicity was generated. However, with each concentration of rIL-2 employed, a much higher level of anti-MOPC-315 cytotoxicity developed in thymocytes from L-PAM-treated MOPC-315 tumor bearers than in thymocytes from normal mice. Consequently, in order to achieve the generation of a comparable level of anti-MOPC-315 cytotoxicity by thymocytes from normal mice as that generated by thymocytes from L-PAM-treated MOPC-315 tumor bearers, a higher concentration of rIL-2 had to be added to the immunization culture. In fact, the lowest concentration of rIL-2 required to enable thymocytes from normal mice to generate a significant level of antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells was 10-fold higher than that required by thymocytes from L-PAM-treated MOPC-315 tumor bearers (i.e., 5.0 versus 0.5 units rIL-2/ml).

Assessment of the Concentration of Exogenous IL-2 Required for the Generation of Antitumor Cytotoxicity in Thymocytes from Untreated MOPC-315 Tumor Bearers or L-PAM-treated Normal Mice in Response to in Vitro Stimulation with MOPC-315 Tumor Cells. Experiments were performed to determine whether thymocytes from untreated MOPC-315 tumor bearers or thymocytes from L-PAM-treated normal mice, like thymocytes from L-PAM-treated MOPC-315 tumor bearers, require less exogenous IL-2 than thymocytes from normal mice for the development of antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells (Fig. 1). While thymocytes from L-PAM-treated MOPC-315 tumor bearers developed a substantial level of antitumor cytotoxicity when immunized in vitro with MOPC-315 tumor cells in the presence of 0.5 units of rIL-2/ml, thymocytes from untreated MOPC-315 tumor bearers or thymocytes from L-PAM-treated normal mice, like thymocytes from untreated normal mice, required the presence of 5.0 units of rIL-2/ml. In addition, whereas thymocytes from L-PAM-treated MOPC-315 tumor bearers developed a much higher level of anti-MOPC-315 cytotoxicity than did thymocytes from normal mice at any concentration of rIL-2 employed, thymocytes from untreated MOPC-315 tumor bearers developed a somewhat higher level of antitumor cytotoxicity than did thymocytes from normal mice only in the presence of 15.0 units of rIL-2/ml. Even then, the level of antitumor cytotoxicity generated by thymocytes from untreated MOPC-315 tumor bearers was lower than that generated by thymocytes from L-PAM-treated MOPC-315 tumor bearers. In contrast to thymocytes from L-PAM-treated MOPC-315 tumor bearers, thymocytes from L-PAM-treated normal mice developed a somewhat lower level of antitumor cytotoxicity than did thymocytes from untreated normal mice in the presence of rIL-2. Thus, thymocytes from L-PAM-treated MOPC-315 tumor bearers are superior, not only to thymocytes from normal mice, but also to thymocytes from untreated tumor bearing mice or L-PAM-treated normal mice in terms of their ability to generate antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells in the presence of low concentrations of exogenous IL-2.

Inability of Exogenous IL-2 Alone to Bring About the Appearance of Anti-MOPC-315 Cytotoxicity in Thymocytes from L-PAM-treated MOPC-315 Tumor Bearers. Experiments were performed to determine whether rIL-2 alone is sufficient to bring about the appearance of anti-MOPC-315 cytotoxicity in thymocytes from L-PAM-treated MOPC-315 tumor bearers, require less exogenous IL-2 than thymocytes from normal mice. Thus, the soluble factors produced by normal spleen cells immunized in vitro with MOPC-315 tumor cells alone are sufficient to enable thymocytes from L-PAM-treated MOPC-315 tumor bearers to develop an antitumor cytotoxic response following stimulation with MOPC-315 tumor cells.
thymocytes from L-PAM-treated MOPC-315 tumor bearers or whether MOPC-315 stimulator tumor cells are also required (Table 2). Thymocytes from L-PAM-treated MOPC-315 tumor bearers, which were superior to thymocytes from normal mice in their ability to mount an antitumor cytotoxic response following in vitro exposure to rIL-2 in the presence of MOPC-315 tumor cells, were unable to develop antitumor cytotoxicity when exposed in vitro to rIL-2 in the absence of MOPC-315 tumor cells. Thus, in vitro exposure to rIL-2 alone at a concentration of 1.5 to 15.0 units/ml is not sufficient to bring about the appearance of anti-MOPC-315 cytotoxicity in thymocytes from L-PAM-treated MOPC-315 tumor bearers and the presence of MOPC-315 tumor cells (or tumor-associated antigens) is also required.

Specificity of the Lytic Activity Expressed by Thymocytes from L-PAM-treated MOPC-315 Tumor Bearers that have been Exposed in Vitro to rIL-2 and MOPC-315 Tumor Cells. Experiments were performed to determine whether thymocytes from L-PAM-treated MOPC-315 tumor bearers that have been exposed in vitro to MOPC-315 tumor cells in the presence of exogenous IL-2 exert an enhanced lytic activity only against the MOPC-315 plasmacytoma or also against other plasmacytomas and even against a thymoma. Specifically, thymocytes from L-PAM-treated MOPC-315 tumor bearers as well as thymocytes from normal mice were exposed in vitro to MOPC-315 tumor cells in the presence of either 5.0 or 15.0 units of rIL-2/ml. Subsequently, the thymocytes were evaluated for their ability to lyse the MOPC-315, MOPC-104E, and RPC-5 plasmacytomas as well as the EL4 thymoma. Fig. 2 illustrates the results obtained when the in vitro immunization was carried out in the presence of 5.0 units of rIL-2/ml. We would like to point out, however, that the same pattern of results was also obtained when the in vitro immunization was carried out in the presence of 15.0 units/ml. As can be seen from Fig. 2, thymocytes from L-PAM-treated MOPC-315 tumor bearers subjected to in vitro immunization with MOPC-315 tumor cells in the presence of rIL-2 exerted a much stronger lytic activity than the in vitro immunized thymocytes from normal mice not only against the MOPC-315 tumor cells but also against the MOPC-104E and the RPC-5 plasmacytomas. On the other hand, the in vitro immunized thymocytes from L-PAM-treated MOPC-315 tumor bearers were in essence not cytotoxic for the EL4 tumor cells. Thus, thymocytes from L-PAM-treated MOPC-315 tumor bearers exhibit, following exposure to MOPC-315 tumor cells plus rIL-2, a greatly enhanced antitumor cytotoxicity not only against the "autochthonous" plasmacytoma but also against other syngeneic plasmacytomas but not against an allogeneic thymoma.

Assessment of the IL-2 Requirements for the Generation of Antitumor Cytotoxicity by Thymocytes from L-PAM-treated MOPC-315 Tumor Bearers upon in Vitro Exposure to Other Plasmacytomas or to a Thymoma. Experiments were performed to determine whether thymocytes from L-PAM-treated MOPC-315 tumor bearers require less exogenous IL-2 than thymocytes from normal mice to develop antitumor cytotoxicity only in response to stimulation with MOPC-315 tumor cells, or also in response to stimulation with other syngeneic plasmacytomas and even with an allogeneic thymoma. For this purpose, thymocytes from L-PAM-treated MOPC-315 tumor bearers or thymocytes from normal mice were cocultured with MOPC-104E, RPC-5, or EL4 tumor cells in the presence of various concentrations of rIL-2 ranging from 0 to 15.0 units/ml. Subsequently, the thymocytes were evaluated for their lytic activity against tumor cells of the same origin as that used for the in vitro immunization. Fig. 3 illustrates the results obtained with the MOPC-104E plasmacytoma. We would like to point out, however, that the same pattern of results was also obtained with the RPC-5 plasmacytoma. As can be seen from Fig. 3, thymocytes from L-PAM-treated MOPC-315 tumor bearers required less rIL-2 than normal thymocytes to develop antitumor cytotoxicity not only in response to stimulation with MOPC-315 tumor cells but also in response to stimulation with MOPC-104E tumor cells. In addition, with each concentration of rIL-2 employed, thymocytes from L-PAM-treated MOPC-315 tumor bearers generated a much higher level of antitumor cytotoxicity than did thymocytes from normal mice in response to stimulation with either MOPC-315 or MOPC-104E tumor cells. In contrast, thymocytes from L-PAM-treated MOPC-315 tumor bearers were not superior, but actually inferior, to thymocytes from normal mice in developing anti-EL4 cytotoxicity in response to stimulation with EL4 tumor cells in the presence of rIL-2 (Fig. 4). Thus, thymocytes from L-PAM-treated MOPC-315 tumor bearers require less exogenous IL-2 than

Table 2: Level of antitumor cytotoxicity exhibited by thymocytes from L-PAM-treated MOPC-315 tumor bearers following in vitro exposure to various concentrations of rIL-2 in the absence of stimulator tumor cells

<table>
<thead>
<tr>
<th>Concentration of rIL-2 (units/ml)</th>
<th>In vitro exposure to MOPC-315</th>
<th>Antitumor cytotoxicity (% of specific ⁵¹Cr release ± SE) exhibited by thymocytes from L-PAM treated tumor bearers</th>
<th>Normal mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>–</td>
<td>4.3 ± 2.3</td>
<td>81.4 ± 0.4</td>
</tr>
<tr>
<td>1.5</td>
<td>–</td>
<td>4.7 ± 2.3</td>
<td>10.8 ± 0.7</td>
</tr>
<tr>
<td>5.0</td>
<td>–</td>
<td>2.1 ± 1.1</td>
<td>10.6 ± 0.8</td>
</tr>
<tr>
<td>15.0</td>
<td>–</td>
<td>3.8 ± 1.7</td>
<td>3.7 ± 1.7</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>3.1 ± 0.5</td>
<td>3.3 ± 1.8</td>
</tr>
<tr>
<td>1.5</td>
<td>+</td>
<td>46.0 ± 3.3</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>5.0</td>
<td>+</td>
<td>87.9 ± 0.3</td>
<td>28.9 ± 1.2</td>
</tr>
<tr>
<td>15.0</td>
<td>+</td>
<td>89.0 ± 1.7</td>
<td>62.9 ± 3.6</td>
</tr>
</tbody>
</table>

*Effector/target cell ratio of 100/1.

* Mice treated with 2.5 mg/kg L-PAM 12 days earlier when they had a 20-22-mm MOPC-315 tumor.
cytotoxicity in response to stimulation not only with MOPC-315 but also with other syngeneic plasmacytomas but not an allogeneic thymoma.

Fig. 3. Level of antitumor cytotoxicity generated by thymocytes from normal mice □, and L-PAM-treated MOPC-315 tumor bearers □ when stimulated in vitro with MOPC-315 or MOPC-104E plasmacytomas. Thymocytes were evaluated for their lytic activity against tumor cells of the same source as that used for the in vitro immunization.

Fig. 4. Level of antitumor cytotoxicity generated by thymocytes from normal mice □, and L-PAM-treated MOPC-315 tumor bearers □ when stimulated in vitro with the MOPC-315 plasmacytoma or the EL4 thymoma. Thymocytes were evaluated for their lytic activity against tumor cells of the same source as that used for the in vitro immunization.

DISCUSSION

The studies presented herein illustrate that treatment of MOPC-315 tumor-bearing mice, but not normal mice, with a low dose of L-PAM brings about a 10-fold reduction in the concentration of exogenous IL-2 required by their thymocytes in order to develop an antitumor cytotoxic response following stimulation with MOPC-315 tumor cells. In addition, at any concentration of exogenous IL-2 employed, thymocytes from L-PAM-treated MOPC-315 tumor bearers develop, upon in vitro stimulation with MOPC-315 tumor cells, a higher level of antitumor cytotoxicity than do thymocytes from untreated MOPC-315 tumor bearers as well as thymocytes from untreated or L-PAM-treated normal mice. The enhanced antitumor cytotoxicity exhibited by thymocytes from L-PAM-treated MOPC-315 tumor bearers, upon in vitro stimulation with MOPC-315 tumor cells plus rIL-2, is evident not only against the MOPC-315 plasmacytoma but also against other syngeneic plasmacytomas, but not an allogeneic thymoma. Finally, thymocytes from L-PAM-treated MOPC-315 tumor bearers require less exogenous IL-2 than thymocytes from normal mice to develop antitumor cytotoxicity in response to stimulation with MOPC-315-associated antigens but not in response to stimulation with an allogeneic thymoma.

We have previously shown that thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not thymocytes from normal mice, can bring about the appearance of enhanced antitumor cytotoxicity when added to the immunization culture of normal spleen cells and MOPC-315 tumor cells (22, 23). Thymocytes from L-PAM-treated MOPC-315 tumor bearers, like thymocytes from normal mice were unable to generate an antitumor cytotoxic response when immunized in vitro with MOPC-315 tumor cells in the absence of normal spleen cells. In the current study we considered the possibility that thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not thymocytes from normal mice, are able to generate an antitumor cytotoxic response in the presence of helper-like factors produced by in vitro immunized normal spleen cells. Here we show that the helper-like factors secreted by normal spleen cells upon in vitro immunization with MOPC-315 tumor cells are sufficient to enable thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not thymocytes from normal mice, to develop an antitumor cytotoxic response following stimulation with the “autochthonous” tumor. The nature of the soluble helper-like factors which are secreted by the MOPC-315-stimulated normal spleen cells has not been elucidated. However, exogenous IL-2 [even though it is probably not the only active factor produced by in vitro immunized normal spleen cells (32)] can substitute for these factors. Accordingly, 0.5 or 1.5 units of rIL-2 per milliliter can bring about the generation of antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells in thymocytes from L-PAM-treated MOPC-315 tumor bearers, but not in thymocytes from normal mice.

As expected (33) the concentration of rIL-2 employed in our study is not sufficient for the generation of LAK cell activity in the thymocytes. Moreover, the concentration of rIL-2 employed (i.e., up to 15.0 units/ml) is not sufficient even to bring about the appearance of anti-MOPC-315 cytotoxicity in thymocytes from L-PAM-treated MOPC-315 tumor bearers, and in vitro exposure to MOPC-315 tumor cells is also required. These results illustrate that the function of the exogenous IL-2 is not just to expand the pool of cytotoxic cells with anti-MOPC-315 reactivity that are already present in the thymuses of the L-PAM-treated MOPC-315 tumor bearers. The antigenic signal
is also required in order to promote the maturation of thymocytes from L-PAM-treated MOPC-315 tumor bearers to the cytotoxic stage.

The reason(s) why thymocytes from L-PAM-treated MOPC-315 tumor bearers require less exogenous IL-2 than thymocytes from normal mice in order to develop an antitumor cytotoxic response following stimulation with MOPC-315 tumor cells is unknown at present. However, several possibilities should be considered. Accordingly, thymocytes from L-PAM-treated MOPC-315 tumor bearers may (a) contain a higher frequency of precursors of cytotoxic T-lymphocytes specific for MOPC-315-associated antigens, (b) produce, upon in vitro stimulation with MOPC-315 tumor cells, some IL-2 or other helper-like factors of their own and consequently require less exogenous IL-2, (c) contain, upon in vitro stimulation with MOPC-315 tumor cells, a higher percentage of cells expressing IL-2 receptors or expressing high affinity or high density IL-2 receptors, and/or (d) contain cells specific for MOPC-315 antigens which are at a more advanced stage of maturation into cytotoxic cells. Experiments are now in progress to evaluate each possibility.

In order to develop antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells, thymocytes from L-PAM-treated MOPC-315 tumor bearers require a 10-fold lower concentration of rIL-2, not only than thymocytes from normal mice, but also than thymocytes from untreated MOPC-315 tumor-bearing mice. The need for a much lower concentration of exogenous IL-2 for the generation of anti-MOPC-315 cytotoxicity by thymocytes from L-PAM-treated MOPC-315 tumor bearers relative to thymocytes from untreated MOPC-315 tumor bearers may be the result of drug-mediated acquisition of new antitumor reactivity and/or unmasking of existing antitumor reactivity. In the first scenario, the L-PAM therapy may enhance the return of antigen-primed mature T-cells from the periphery into the thymus in a similar way to the enhanced reentry of T-lymphocytes into the thymus following irradiation (34). In the second scenario, although MOPC-315 tumor cells apparently do not metastasize into the thymus (23), MOPC-315 tumor-associated antigens, like nontumor antigens administered systemically into normal mice (35), are expected to enter the thymuses of the MOPC-315 tumor bearers and cause in situ antigenic stimulation. Enhanced anti-MOPC-315 reactivity is not evident, however, in the thymuses of untreated MOPC-315 tumor bearers as a consequence of the simultaneous stimulation of suppressor cell activity in a comparable way to that reported by Hancock et al. (36) in the thymuses of mice at an advanced stage of P815.X2 tumor growth. The antitumor immune reactivity of thymocytes from MOPC-315 tumor bearers can be realized in this scenario if the low dose of L-PAM eliminates (or reduces) the effectiveness of the suppressive activity from the thymuses of the MOPC-315 tumor bearers in a comparable way to that in which it eliminates the effectiveness of suppressor cell activity from the spleens of the MOPC-315 tumor bearers (10, 22).

In order to develop antitumor cytotoxicity in response to stimulation with MOPC-315 tumor cells, thymocytes from L-PAM-treated MOPC-315 tumor bearers require less exogenous IL-2, not only than thymocytes from normal mice, but also than thymocytes from L-PAM-treated normal mice. These results illustrate that treatment of mice with L-PAM alone is not sufficient to enable their thymocytes to develop an antitumor cytotoxic response in the presence of lower concentrations of exogenous IL-2. On the contrary, treatment of mice with a low dose of L-PAM reduces the ability of their thymocytes to develop, in the presence of exogenous IL-2, an antitumor cytotoxic response against an antigen not encountered previously by the intact mouse. This is evident in our study from (a) the reduced level of anti-MOPC-315 cytotoxicity generated by thymocytes from L-PAM-treated normal mice relative to thymocytes from untreated normal mice, and (b) the reduced level of anti-EL4 cytotoxicity generated by thymocytes from L-PAM-treated MOPC-315 tumor bearers relative to thymocytes from untreated normal mice. Thus, a low dose of L-PAM, which when administered to MOPC-315 tumor bearers leads to enhancement in the ability of their thymocytes to generate antitumor cytotoxicity in response to stimulation with MOPC-315-associated antigens plus exogenous IL-2, brings about suppression in the ability of thymocytes to develop antitumor cytotoxicity in the presence of exogenous IL-2 in response to stimulation with antigens not previously encountered. This may be due to a greater sensitivity to the toxic effects of L-PAM of thymocytes involved in the generation of a primary antitumor cytotoxic response relative to cells involved in the generation of a cytotoxic response against an antigen previously encountered by the mouse. Alternatively, it is possible that both groups of thymocytes are equally sensitive to the toxic effects of the low dose of L-PAM. However, L-PAM-induced reduction in the frequency of cells involved in the generation of anti-MOPC-315 cytotoxicity in the thymuses of MOPC-315 tumor bearers is less detrimental than a similar magnitude of reduction in the frequency of such cells in the thymuses of normal mice in terms of the ability to mount an anti-MOPC-315 cytotoxic response. This can happen, for example, if the frequency of cytotoxic T-lymphocyte precursors specific for MOPC-315 associated antigens is increased in the thymuses of MOPC-315 tumor bearers during tumor progression.

It should be stressed at this stage, that the thymocytes from L-PAM-treated MOPC-315 tumor bearers employed in our studies were derived from mice that 2 to 4 days earlier completed the eradication of a large s.c. MOPC-315 tumor burden. The eradication of the tumor occurs in such mice primarily through the action of T-cell-dependent antitumor immunity, while the drug itself has very little direct antitumor effect (2). The T-cells from such mice display direct cytotoxic activity against MOPC-315 cells bearing, as well as those lacking, surface immunoglobulins and coexisting within the same tumor nodule (37). In this regard, it is interesting that thymocytes from L-PAM-treated MOPC-315 tumor bearers can generate enhanced antitumor cytotoxicity which is effective not only against cells bearing the MOPC-315 idiotype, but also against plasmacytomas lacking the MOPC-315 idiotype on their surface (i.e., the MOPC-104E and RPC-5). This raises the question of whether the thymus plays any role in the ability of the cytolytic antitumor immunity, which emerges shortly after low-dose L-PAM therapy of mice bearing a large MOPC-315 tumor, to reject the enormous tumorigenic burden remaining after clearance of the drug from the circulation. In addition, since the thymus may function as a repository of memory T-cells (38), the thymus may contribute to the long-lasting ability of L-PAM-treated MOPC-315 tumor bearers to reject a challenge with as many as 1 x 10^8 MOPC-315 tumor cells which corresponds to 30,000-fold the minimal lethal tumor dose for normal mice (39).

REFERENCES


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ENHANCEMENT OF THYMIC ANTITUMOR IMMUNE REACTIVITY


Interleukin 2 Requirement for the in Vitro Generation of Antitumor Cytotoxicity by Thymocytes from Melphalan-cured MOPC-315 Tumor Bearers

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