Secondary in Vitro Lymphocyte-proliferative Responses to Syngeneic Plasma Cell Tumors

P. J. J. Boyer and J. L. Fahey

Department of Microbiology and Immunology, UCLA School of Medicine, University of California, Los Angeles, California 90024

SUMMARY

Two characteristics of immune responses to weakly immunogenic plasma cell tumors were demonstrated in this study. (a) Elevated lymphoproliferative responses following in vivo inoculation of sublethal doses of plasma cell tumors were detected by a mixed lymphocyte-tumor interaction (MLTI) assay. Specificity of elevated MLTI responses correlated with resistance to in vivo tumor challenge. These responses were dependent upon the presence of thymus-derived cells. (b) Spleen and lymph node cells showed markedly different patterns of MLTI activity following plasma cell tumor immunization. Spleen cell responses were depressed for the first 30 days postimmunization, whereas lymph node cells showed augmented MLTI reactivity immediately after immunization. These observations indicated that lymphocyte populations from different lymphoid organs may show widely dissimilar responses in vitro at the same point in the in vivo development of tumor resistance.

INTRODUCTION

In vitro lymphocyte proliferation, as measured by syngeneic MLTI assays, has been proposed as a measurement of syngeneic antitumor immunity (2, 5). Several investigators have reported that proliferative responses to syngeneic tumor cells are detectable in vitro only after successful in vivo immunization against tumor-associated antigens and cannot be elicited from lymphoid cells of unimmunized animals (5, 16, 24). Others have reported blastogenic responses to tumor cells without known prior sensitization (9, 20, 25). Previous investigations in our laboratory have shown that lymphoid cells from both unimmunized and immunized BALB/c mice demonstrate proliferative responses upon exposure to syngeneic PCT cells in vitro. The detection of MLTI responses by lymphocytes from unimmunized animals allows a positive background against which the effects of tumor immunization may be investigated. In addition, evidence for the presence of weakly immunogenic tumor-associated antigens on the BALB/c PCT has been provided by transplantation resistance studies in syngeneic mice (10, 17, 18, 22, 27, 31, 32). These data permit comparison of MLTI specificity experiments with in vivo resistance to tumor growth.

Syngeneic antibody responses to at least 2 types of PCT cell surface antigen have been detected serologically. One type of PCT antigen is apparently shared by all BALB/c PCT’s investigated thus far (7); the other type includes those antigens characteristic of 1 or several individual PCT lines, but which are not commonly shared (3, 11, 15). Although tumor cell surface structures stimulating antibody responses may be different from those eliciting proliferative or cellular responses, it is of interest to determine whether postimmunization alterations in MLTI activity would (a) reflect specific responses to individual tumor-associated antigens; (b) show altered reactivity to all syngeneic PCT’s, thus indicating response to shared PCT antigens; or (c) show broad nonspecific changes in reactivity to all syngeneic tumors.

In these studies, we have evaluated the specificity and kinetics of postimmunization MLTI responses for their relevance to specific antitumor immunity in vivo.

MATERIALS AND METHODS

Mice. BALB/c and C57BL/6 mice used in these studies were 8- to 10-week-old females obtained from Simonsen Laboratories (Gilroy, Calif.) and maintained subsequently in our animal facilities.

Tumor Lines. All BALB/c PCT’s used in these experiments were maintained in BALB/c mice as solid s.c. tumors. Prior to use in experiments, the tumors were transferred to the ascitic form in which they were maintained for 2 to 15 generations as sources of cells for routine use. LPC-1, MOPC-315, MOPC-104E, and ABE-1 were obtained through the courtesy of Dr. M. Potter.

Immunization of Animals. Groups of mice were inoculated s.c. with $10^4$ to $10^5$ viable PCT cells as indicated in individual experiments. Age-matched mice were used for unimmunized controls in all experiments. Only mice with no detectable palpable tumors were used for in vitro testing.

Cell Suspensions. Mice were sacrificed by cervical dislocation. Spleens and lymph nodes were removed aseptically, teased apart in BSS, and passed through sterile Nitex screens (Tetko, Inc., Elmsford, N. Y.) to remove clumps. Lymph node cells were from pooled axillary, brachial, cervi-

1 This work was supported by NIH Grant CA 12800.

2 Supported from Grant 5T01 Al 00431 in Clinical and Fundamental Immunology. To whom requests for reprints should be sent, at Department of Microbiology and Immunology, Center for the Health Sciences, School of Medicine, University of California, Los Angeles, Calif. 90024.

3 The abbreviations used are: MLTI, mixed lymphocyte-tumor interaction; PCT, plasma cell tumor; BSS, balanced salt solution; RPMI, Roswell Park Memorial Institute; FCS, fetal calf serum; T-cell, thymus-derived cell; MSV, murine sarcoma virus.

Received September 8, 1975; accepted January 8, 1976.
treated for 5 mm at room temperature with freshly prepared Gey's solution (14) to which NH₄Cl (35.0 g/liter) and gelatin (25.0 g/liter) (Difco Laboratories, Inc., Detroit, Mich.) had been added to Solution A, to remove RBC. After treatment, the cells were washed twice and counted. Only suspensions with greater than 90% viable cells as assessed by trypan blue exclusion were used for experiments.

Ascitic fluid containing tumor cells was removed aseptically from the peritoneal cavity of tumor-bearing mice 8 to 12 days after tumor transfer, washed, and treated as described for lymphoid cell suspensions prior to use.

Mitomycin C Treatment of Stimulating Cells. Stimulating cells were treated with Mitomycin C by incubating 1 x 10⁶ cells in 5 ml of RPMI Medium 1640 containing 1% glutamine, 1% penicillin-streptomycin-Fungizone (Grand Island Biological Co., Grand Island, N. Y.), 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, and Mitomycin C (25 µg/ml) (Sigma Chemical Co., St. Louis, Mo.) at 37° in 5% CO₂ in air for 45 min with intermittent shaking. The cells were then washed 4 times before use. This treatment was shown to block [³H]thymidine incorporation uniformly in the PCT used (2).

MLTI Assay. Triplicate cultures of 1 x 10⁶ responding cells and 2 x 10⁶ stimulating cells in 2 ml of RPMI Medium 1640 medium supplemented with 10% FCS (Reheis Chemical Co., Chicago, Ill.; Lot L23504 was used for all experiments), 1% glutamine, 1% penicillin-streptomycin-Fungizone (Grand Island Biological Co.), 5 x 10⁻³ M 2-mercapto-ethanol (Calbiochem, Los Angeles, Calif.) and buffered with 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer (Grand Island Biological Co.) and sodium bicarbonate to pH 7.2 (FCS-supplemented RPMI Medium 1640) were incubated in loosely capped plastic culture tubes (Falcon 3033) at 37° in a humidified atmosphere of 5% CO₂ in air for 96 hr unless otherwise indicated. [methyl-³H]Thymidine (Schwarz/Mann, Orangeburg, N. Y.; specific activity, 14.8 Ci/m mole) was added to a final concentration of 2.0 µCi/ml, and the cultures were incubated for an additional 16 hr. The cells were then washed with BSS, and the acid-insoluble material was precipitated with cold 5% trichloroacetic acid, collected on glass fiber filters (Millipore Corp., Bedford, Mass.; No. AP2502500), and dried. The dried filters were placed in glass scintillation vials with 10 ml of scintillation liquid containing 160 ml of Spectrofluor POPOP (Amer sham/Searle Corp., Arlington Heights, Ill.) per 3.79 liters of toluene, and the samples were counted in a Beckman LS-330 liquid scintillation spectrometer.

Responding cell controls included: (a) 1 x 10⁶ responding cells cultured alone, and (b) 1 x 10⁶ responding cells cultured with 2 x 10⁶ Mitomycin C-treated responding cells.

Anti-θ Treatment of Responding Cell Populations. AKR anti-θ C3H serum was prepared according to the method of Raff (21), heat inactivated by incubation at 56° for 1 hr, and adsorbed with ABE-i, BALB/c lymphoma cells at a packed cell:serum volume ratio of 1:4 for 1 hr at 4° to remove non-θ activity. Normal rabbit serum that had been absorbed with normal BALB/c spleen cells at a packed cell:serum volume ratio of 1:4 for 1 hr at 4° and diluted 1:3 in Veronal buffer was used as a source of complement. Responding cell suspensions were incubated with a sufficient volume of antiserum to produce maximal killing or an equal volume of control serum for 30 min at 37°; then they were centrifuged and the supernatant was discarded. The complement source was then added, and the cells were resuspended and incubated for an additional hr at 37°. After incubation, the cells were washed 2 times with BSS and suspended in FCS-supplemented RPMI Medium 1640.

Following treatment with anti-θ serum and complement, remaining viable cells were counted by trypan blue exclusion and, as an additional measure of cell depletion, tested for their ability to respond to allogeneic stimulation, phytohemagglutinin, and lipopolysaccharide. Mitogen stimulation was assessed by incubation of 1 x 10⁶ cells in 1 ml of FCS-supplemented RPMI Medium 1640 in the presence of either lipopolysaccharide (10 µg/ml) (Escherichia coli 055:B5; Difco) or a 1:100 dilution of phytohemagglutinin (Wellcome Research Laboratories, Beckenham, England), and [³H]thymidine incorporation was determined as previously described after 60 hr in culture. Allogeneic stimulating cells (C57BL/6 spleen cells (H-2b)) were treated with Mitomycin C in the same manner as syngeneic stimulating cells and allogeneic cultures were harvested at the same time.

RESULTS

Immunization of Mice. BALB/c mice that had been inoculated with sublethal doses of viable syngeneic PCT cells resisted challenge with subsequent lethal doses of tumor
cells. By in vivo immunization and cross-challenge methods, some PCT's have been found to express individual tumor-associated transplantation antigens, while others appear to express cross-reacting antigens. Groups of age-matched BALB/c mice were given low doses of either MOPC-104E, MOPC-315, or LPC-1 and later challenged with lethal doses of various tumors to determine the in vivo specificity of resistance to challenge (Table 1). The results indicate that animals immunized with MOPC-104E showed resistance to challenge with MOPC-104E and LPC-1 but not to other tumors tested. Animals immunized with MOPC-315 showed resistance to only MOPC-315, and mice immunized with LPC-1 were resistant to both LPC-1 and MOPC-104E. These findings indicate that LPC-1 and MOPC-104E share a common tumor-associated antigen involved in the in vivo resistance to tumor growth and not present on the other tumors used.

Changes in MLTI Reactivity following in Vivo Immunization. For determination of the extents of immunization on in vitro MLTI reactivity, responses of spleen and lymph node cells were tested at various times following sublethal dose immunization and compared to the responses of spleen and lymph node cells from unimmunized, age-matched mice tested at the same time. Chart 1 shows the results of experiments in which mice were inoculated with 10⁶ viable LPC-1 cells and tested for MLTI activity against LPC-1 and MOPC-315 at various times after the immunizing injection. Immunized cell responses are expressed as the percentage of unimmunized cell responses tested on the same day as described in "Materials and Methods" to exclude changes due to day to day variation in the assay itself. Spleen cells from immunized animals demonstrated depressed responses as compared to the responses of unimmunized animals for approximately 30 days followed by a rapid increase in activity which reached a plateau at 50 to 60 days and declined to unimmunized cell levels by 97 days postimmunization (Chart 1).

Table 1

<table>
<thead>
<tr>
<th>Tumor challenge</th>
<th>Control</th>
<th>LPC-1</th>
<th>MOPC-104E</th>
<th>MOPC-315</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>0/9⁴</td>
<td>17/18</td>
<td>4/4</td>
<td>0/8</td>
</tr>
<tr>
<td>Immunizing tumor</td>
<td>0/9</td>
<td>10/10</td>
<td>4/4</td>
<td>0/5</td>
</tr>
<tr>
<td>MOPC-315</td>
<td>0/9</td>
<td>0/14</td>
<td>0/4</td>
<td>13/14</td>
</tr>
<tr>
<td>MOPC-21A</td>
<td>0/6</td>
<td>0/8</td>
<td>ND⁵</td>
<td>0/5</td>
</tr>
<tr>
<td>Meth A</td>
<td>0/11</td>
<td>0/8</td>
<td>ND</td>
<td>0/5</td>
</tr>
</tbody>
</table>

⁴ Mice were challenged by s.c. injection of 5 x 10⁶ viable tumor cells.
⁵ No treatment prior to challenge.
⁶ Mice were given s.c. injections of 10⁶ viable cells 2 times 2 weeks apart and challenged 3 weeks after the final immunizing inoculation. Only animals that had not developed tumors during the course of immunization were challenged.
⁷ Number of mice surviving with no palpable tumor at 100 days/number of mice inoculated with challenge tumor. Data pooled from 3 experiments.

Only responses to the immunizing PCT were depressed between 12 and 30 days postimmunization, while responses to other non-cross-reacting PCT (MOPC-315) and allogeneic stimulation (data not shown) remained within unimmunized cell ranges (Chart 1). During the 1st 12 days following immunization, however, specificity of this depression could not be established. In 3 series of experiments, spleen cells from LPC-1-immunized animals were tested 22 times between 1 and 12 days following immunization. In 11 of 22 tests, the spleen cells exhibited depressed responses to all stimulating cells, indicating nonspecific depression. In the other 11 of 22 tests, the responses to LPC-1 were depressed while responses to other tumors were not significantly different from those of unimmunized spleen cells.

Lymph node cells from LPC-1-immunized animals (in contrast to spleen cells) demonstrated consistently elevated reactivity to LPC-1, which reached maximum levels at 30 to 40 days and declined to unimmunized levels by 97 days (Chart 1). Immediately following immunization, responses to MOPC-315 and other stimuli appeared slightly elevated but returned to unimmunized cell levels approximately 12 days postimmunization. In summary, the specific MLTI responses of lymph node cells from immunized animals increased during the 1st 30 days postimmunization while spleen cells from the same animals tested at the same time showed a depressed response. After 30 days postimmunization, however, both spleen and lymph node cells demonstrated elevated MLTI responses to the immunizing tumor.

To exclude the possibility that elevated MLTI responses might be due to kinetic shifts in reactivity rather than actual elevations in responding cell activity, studies were undertaken to determine MLTI activity at various times after the initiation of the cultures. Six experiments (1-of which is shown in Chart 2) indicated that responding cells from immunized mice reach maximum levels of tritiated thymidine incorporation somewhat earlier than did cells from other tumors used.
nonimmunized mice, but this shift does not account for the elevations observed in response to the immunizing PCT.

Specificity of MLTI Reactivity. To determine whether the specificity of transplantation resistance observed in vivo would be reflected in vitro, spleen cells from immunized mice were tested for MLTI activity using various syngeneic and allogeneic stimulating cells. Data shown in Table 2 indicate a correlation between the specificity of in vivo resistance to tumor challenge and elevated MLTI responses in vitro. Mice that had been immunized with LPC-1 showed increased responses to LPC-1 and MOPC-104E but no significant increases to other syngeneic or allogeneic stimulation. Similarly, mice inoculated with MOPC-104E showed increased responses to both MOPC-104E and LPC-1, but not to other stimulation. Mice that had been immunized with MOPC-315 showed only elevated responses to MOPC-315. Elevated responses to non-PCT tumors, allogeneic stimulation, or mitogens were not observed.

Dose-Response Kinetics of MLTI Reactivity. The effect of immunizing tumor cell dose on changes in MLTI reactivity following immunization was assessed by inoculation of age-matched mice with 10³ to 10⁵ viable LPC-1 cells and the MLTI activity of spleen cells tested at various times after inoculation. Spleen cell responses as shown in Chart 3 were depressed at all doses for approximately 30 days after immunization. Subsequently, the major change in reactivity was quantitative, i.e., the magnitude of the MLTI response was dependent upon the size of the immunizing tumor dose. Specificity was not altered by the different tumor doses.

Nature of the Responding Cells. To determine whether the elevated MLTI responses of lymphoid cells from immunized mice were dependent upon the presence of ß-bearing T-derived cells, spleen cells from animals that had been immunized 55 days previously were treated with anti-ß serum and complement or control serum and complement as previously described. This treatment markedly reduced the in vitro reactivity to the immunizing PCT as shown in Table 3A. These results indicated that the elevated responses postimmunization were primarily dependent upon the presence of T-cells in the spleen preparation.

Spleen cells from mice 13 days postimmunization demonstrated negligible MLTI responses to the immunizing PCT cells (Table 3B). Treatment of these cells with anti-ß serum and complement did not restore MLTI responses, although responses to other T-cell stimuli were reduced as expected.
DISCUSSION

In this study, we observed that lymphocytes from mice immunized by sublethal dose inoculation of viable syngeneic PCT cells demonstrated both elevated and depressed MLTI responses to the immunizing PCT at different times after injection. The observed elevations of [3H]thymidine incorporation were not due to the presence of tumor cells in lymph nodes or spleens. Histological examination of responding cell preparations failed to reveal malignant plasma cells. Further, [3H]thymidine incorporation of lymphoid cells from immunized and normal mice cultured in the absence of stimulating tumor cells was the same.

Several types of cell surface antigen have been demonstrated on BALB/c PCT that could be responsible for the stimulation of syngeneic lymphoid cells.

The specificity of elevations in MLTI activity exhibited by lymphocytes from immunized mice paralleled in vivo protection, indicating that the same tumor cell surface antigens might be involved in both phenomena. Correlation between antigen-specific proliferative reactivity in vitro and resistance to tumor induction has been reported in an avian oncornavirus system in which animals were immunized and tested against live homologous or heterologous virus rather than tumor cells (16).

Serological studies (3, 7, 11, 15) indicate that these tumors possess at least 2 groups of cell surface antigens to which syngeneic mice can respond immunologically: (a) shared antigens which appear to be common to all BALB/c PCT; and (b) individual antigens which are found on 1 or several tumors but not all PCT's. Although serologically defined antigens may be different from those eliciting protective resistance to tumor challenge, in vivo specificity studies reported here and by other workers (17, 22, 27, 32) indicate that protective immune responses in vivo are directed against cell surface antigens which would be classified as individual (rather than shared) antigens. Similarly, MLTI-stimulating PCT antigens appear to be primarily individual rather than common antigens. In studies conducted in parallel with the present study, serological methods showed that all PCT's used shared a common antigen(s) (unpublished data). Two of the PCT's, LPC-1 and MOPC-104E, were also found to share additional cross-reacting cell surface determinants not expressed by the other PCT's tested. Cross-reactivity between LPC-1 and MOPC-104E could also be demonstrated in in vivo protection studies in confirmation of the earlier transplantation resistance studies of McCoy et al. (17). MLTI specificity experiments also reflected this cross-reactivity between LPC-1 and MOPC-104E, indicating detection of individual tumor-associated antigens that may be identical to those detected by serological and in vivo methods.

The role of common PCT antigens in MLTI stimulation is not yet established. Studies by Herberman and Aoki (7)

Table 3

<table>
<thead>
<tr>
<th>Pretreatment of responding cells</th>
<th>Control* (responding cells alone)</th>
<th>Phytohemagglutinin*</th>
<th>Lipopolysaccharide*</th>
<th>Control* CS57BL/6 spleen cells (allogeneic)</th>
<th>LPC-1*</th>
<th>MOPC-315*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Responding cells taken 55 days after s.c. inoculation of 10^9 viable LPC-1 tumor cells</td>
<td>1,206 ± 51^a</td>
<td>23,884 ± 1,786^a</td>
<td>10,089 ± 314^a</td>
<td>1,107 ± 132</td>
<td>6,781 ± 342^a</td>
<td>5,093 ± 95^a</td>
</tr>
<tr>
<td>None</td>
<td>1,091 ± 76</td>
<td>10,281 ± 289^a</td>
<td>1,547 ± 693</td>
<td>2,496 ± 133</td>
<td>1,107 ± 132</td>
<td>6,781 ± 342^a</td>
</tr>
<tr>
<td>Anti-γ and C′</td>
<td>730 ± 39</td>
<td>11,052 ± 179^a</td>
<td>1,266 ± 157</td>
<td>6,840 ± 157^a</td>
<td>5,670 ± 117^a</td>
<td>2,574 ± 140^a</td>
</tr>
<tr>
<td>Normal CS57BL/6 serum and C</td>
<td>2,489 ± 53</td>
<td>16,572 ± 177^a</td>
<td>792 ± 57</td>
<td>8,51 ± 27</td>
<td>1,107 ± 132</td>
<td>6,781 ± 342^a</td>
</tr>
</tbody>
</table>

| B. Responding cells taken 13 days after s.c. inoculation of 10^9 viable LPC-1 tumor cells | 1,964 ± 93 | 52,001 ± 691^a | 4,402 ± 280^a | 691 ± 51 | 2,849 ± 78^a |
| None | 2,489 ± 53 | 16,572 ± 177^a | 792 ± 57 | 8,51 ± 27 | 1,107 ± 132 | 6,781 ± 342^a | 5,093 ± 95^a | 2,722 ± 55^a |

* Mitogen-stimulated cultures and controls harvested at 60 hr.
* Two × 10^7 Mitomycin C-treated stimulating cells added to cultures. Experimental cultures and controls harvested at 97 hr.
* Mean ± S.D. of triplicate cultures.
* p < 0.005 as compared to unstimulated control values.
* Anti-γ treatment and absorption as described in "Materials and Methods."
indicate that among the shared PCT antigens to which syngeneic hosts can respond immunologically is a cell surface antigen indistinguishable from the alloantigen, PC.1, characterized by Takahashi et al. (26) and present on normal plasma cells of certain strains of mice. The biological significance of PC.1 and similar cell surface antigens is not known. They may be involved or possibly required for the induction of syngeneic proliferative responses, particularly in the case of responses by cells from unimmunized animals. Several laboratories that have reported strong proliferative responses to other syngeneic tumor cells following immunization have been unable to detect MLTI responses with cells from unimmunized animals (5, 16, 24), indicating that the presence of immunologically recognizable tumor-associated antigens on the syngeneic stimulating cells is not sufficient for the in vitro induction of proliferation by unprimed cells. In the case of the BALB/c PCT, the observation that approximately 93% of 12-week-old "normal" BALB/c mice have circulating cytotoxic antibody directed against a cell surface antigen indiscernible from PC.1 (7) suggests that "natural" immunological priming against the PC.1-like shared PCT antigen may fulfill cellular requirements for subsequent proliferative responses to individual tumor-associated antigens, even by unimmunized "normal" lymphoid cells.

Viral antigens may also be involved in the induction of proliferative responses. The differentiation antigen, PC.1, has been associated with a viral envelope antigen (xVEA) (1, 7). In addition, the Gross leukemia cell surface antigen and the Gross viral envelope antigen have also been found on some PCT's (1, 8). All BALB/c PCT examined thus far contain intracisternal A-type particles (12, 30), and production of C-type particles by several lines has been reported (11, 28, 29). Krueger et al. (11) have further observed that immunized or tumor-bearing syngeneic mice produce humoral antibodies directed against viral antigens on or within PCT cells indicating that virus-related antigens may also stimulate detectable immune responses in this system.

Attempts have been made to relate syngeneic MLTI responses to other parameters believed to be involved in the immunological control of syngeneic tumor growth. Specificity of immune lymphocyte MLTI responses and correlation with in vitro-generated cell-mediated cytotoxicity have been reported by Glaser et al. (5) using the strongly immunogenic Gross virus-induced lymphoma, (C58NT)D, in syngeneic rats. Senik et al. (24), using a MSV-induced tumor system, have also reported that the specificity of MLTI activity following immunization correlated well with the specificity of direct cell-mediated cytotoxicity assessed by a 51Cr release assay, indicating that similar or identical antigens on the MSV-induced tumors were detected by MLTI and cell-mediated cytotoxicity. MLTI-responding cells, however, had no cytotoxic activity in the 51Cr release assay (24), indicating either detection of different reactive cell populations or different stages of differentiation of the same cell population by the 2 assay methods. Later studies with MSV-induced tumors (19) have shown that the cell population(s) responsible for cytotoxicity in the 51Cr release assay are not the same as those detected by a microcytotoxicity assay method. Only T-cells were found to be active in the 51Cr release assay, while both T- and non-T-effector cells were demonstrated in the microcytotoxicity assay. Additionally, using a microcytotoxicity assay only, Lamon et al. (13) have shown that different lymphocyte subpopulations function as cytotoxic effector cells at different times during the induction, development, and regression of Moloney sarcoma virus-induced tumors. These findings strongly suggest that 2 or more lymphocyte subpopulations may react to the same cell surface determinants on syngeneic tumors and that not all reactive populations may generate detectable MLTI activity.

Although elevated MLTI activity may indeed be indicative of an ongoing state of cellular immunity as postulated by Glaser et al. (5), these responses are not continuously observed during the course of a secondary immune response to syngeneic tumor cells. Glaser et al. (5) observed that significant MLTI reactivity against (C58NT)D cells occurs only at a time after direct cell-mediated cytotoxicity measured by a 51Cr release assay is no longer detectable. The appearance of MLTI reactivity following decline of detectable 51Cr release cytotoxicity using spleen cells from mice that had been immunized with irradiated Friend- or Rauscher virus-induced leukemia cells or Friend leukemia virus has also been reported by Dean et al. (4). On the other hand, Senik et al. (24), studying spleen cells from MSV-infected mice, found MLTI reactivity only prior to detection of cytotoxic cells. However, these mice developed tumors concomitantly with the disappearance of MLTI reactivity as opposed to other tumor systems studied in which the animals did not develop progressively growing tumors. In the (C58NT)D system, Glaser et al. (6) have demonstrated that, while spleen cells from regressor rats have significant activity in the MLTI assay, spleen cells from rats with progressively growing tumors were unresponsive due to the presence of a suppressor cell population. The kinetics of augmented spleen cell MLTI responses to PCT following immunization most closely resembled those reported by Glaser et al. (5), although specifically elevated MLTI responses to PCT were not observed until 30 days following immunization as compared to 14 days in the (C58NT)D system, and cell-mediated cytotoxicity data are not yet available for comparison.

The kinetics of MLTI reactivity following immunization shows that spleen but not lymph node cell MLTI responses are depressed during the 1st 30 days. As noted, the initial depression (1st 12 days) is frequently nonspecific. After 12 days, however, responses to other allogeneic and syngeneic stimulation returned to normal levels and only responses to the immunizing tumor remained depressed. This depression reflects either an absence of cells in the spleen that can be stimulated by the immunizing PCT at this time or the effect of suppressive mechanisms that limit proliferation of tumor antigen-reactive cells in the spleen. Preliminary experiments in which the addition of cells from immunized mice to cultures of cells from unimmunized mice specifically reduced MLTI activity (P. J. J. Boyer and J. L. Fahey, unpublished results) suggest the action of lymphocyte-dependent suppressive mechanisms operative in these cultures which may reflect a limited period of in vivo tumor growth following injection of viable immunizing cells. The disparity be-
between the activities of spleen and lymph node cells during the 1st 30 days after immunization most likely reflects either the presence of different cell populations in the 2 lymphoid organs or different regulatory conditions that could modify the activity of lymphoid cells in these environments, neither of which possibilities is mutually exclusive. Differences between the activity of spleen and lymph node cells have been observed in other syngeneic systems (23). MLTI responses may be depressed, elevated, or at normal levels at various times postimmunization depending on the lymphoid organ chosen for testing, which could preclude interpretation of negative MLTI data in kinetically undefined situations as being indicative of an absence of cellular responsiveness to tumor-associated antigens.

ACKNOWLEDGMENTS

The authors wish to acknowledge the expert technical assistance of Barbara Ikijeri and Francine Orner.

REFERENCES

Secondary *in Vitro* Lymphocyte-proliferative Responses to Syngeneic Plasma Cell Tumors

P. J. J. Boyer and J. L. Fahey

*Cancer Res* 1976;36:1492-1498.