Chemotherapy and Immunotherapy of L1210 Leukemic Mice with Antigenic Tumor Sublines

Angelo Nicolin, Gianfranco Canti, Ornella Marelli, Fulvia Veronese, and Abraham Goldin

Institute of Pharmacology, Via Vanvitelli 32, Milan, Italy [A. N. G. C., O. M., F. V.], and Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20205 [A. G.]

ABSTRACT

Tumor cells, treated in vivo with anticancer compounds, may acquire new antigenic specificities in addition to any original antigens associated with parental tumors. Treatment of mice carrying the parental leukemias L1210 Ha or L1210 Cr with leukemia cells antigenically altered by treatment with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (L1210 Ha/DTIC and L1210 Cr/DTIC, respectively) was essentially ineffective in prolonging the life span of the animals. However, synergic therapeutic activity was exhibited by administration of L1210 Ha/DTIC cells plus 1,3-bis(2-chloroethyl)-1-nitrosourea in the treatment of the moderately immunogenic L1210 Ha leukemia and by the combination of L1210 Cr/DTIC cells and lymphocytes immune to L1210 Cr/DTIC administered with 1,3-bis(2-chloroethyl)-1-nitrosourea in the treatment of the low immunogenic L1210 Cr leukemia. Early and advanced L1210 Cr-bearing mice showed marked increases in survival time and a significant number of tumor-free survivors on treatment with cyclophosphamide followed by transfer of lymphocytes immune to L1210 Cr/DTIC cells. When parental tumor cells were used as the immunogen, the therapeutic effect was diminished.

Thus, in the current investigation, although immunotherapy per se was essentially ineffective, the immunocamtherapy modes used were successful in markedly increasing the survival time of leukemic animals and resulted in an incidence of cures.

INTRODUCTION

Increased responsiveness (collateral sensitivity) of drug-resistant tumors to treatment with a structurally unrelated antineoplastic agent has been observed in the course of chemotherapeutic treatment of experimental (2, 11, 12) and human cancer (10). Moreover, in a syngeneic system, in a number of instances, drug-resistant tumor cells have been found to be more immunogenic than the parental, untreated tumor cells (19). It was therefore suggested that synergic activity between host immune reaction to drug-resistant tumors and effective chemotherapy could provide a basis for the increased host survival (1, 10, 18, 20). These findings prompted in vivo studies designed to increase the antigenic properties of experimental tumor cells by pharmacological means (1, 18). A series of in vivo and in vitro investigations established that drug-treated tumor sublines could be so altered that they were rejected by the original compatible hosts and that drug-induced antigens were maintained indefinitely even after withdrawal of drug administration (14, 15).

In addition to pharmacologically induced antigens, TAA have been detected on the surface of drug-treated cells (15, 17). Animal immunization with viable drug-treated cells elicited stronger host resistance to the challenge of parental tumor than did inactivated parental cells (21).

In this case, highly immunogenic drug-treated lymphomas were similar to tumors capable of sensitizing the host against TAA of cross-reacting syngeneic neoplasms, as described previously for leukemias generated by the Friend-Moloney-Rauscher murine leukemia virus group (5, 7).

The current studies were conducted with 2 lines of leukemia L1210, the moderately immunogenic L1210 Ha line and the weakly immunogenic LCr tumor. Viable highly immunogenic cells of the 2 leukemia lines obtained following in vivo treatment with DTIC were used for host sensitization after tumor challenge. In addition, adoptive transfer of lymphocytes immune to the DTIC-treated leukemic cells was used alone or in combination with chemotherapy in mice bearing the parental leukemias. The experiments indicate that improvement of therapeutic response to anticancer drugs may be obtained in leukemic mice when active or adoptive immunotherapy is included in the treatment regimen.

MATERIALS AND METHODS

Animals and Tumors. Inbred DBA/2Cr and hybrid BALB/c x DBA/2Cr F, (CD2F,) mice of both sexes, 8 to 10 weeks old, were obtained from the Charles River Breeding Laboratory, Calco, Italy.

LHa provided by Dr. E. Mihich, Roswell Park Memorial Institute, Buffalo, N. Y., and LCr from the National Cancer Institute, Bethesda, Md., were passed weekly i.p. in DBA/2 mice.

The antigenic sublines LHa/DTIC and LCr/DTIC were developed as described previously with minor modifications (1). Briefly, 10^6 cells were injected i.p. into CD2F, mice, and the animals were treated for 7 consecutive days with DTIC (100 mg/kg/day i.p.) starting 1 day after tumor challenge. When ascites tumor developed in the DTIC-treated mice, tumor cells were collected, and 10^6 cells were transplanted i.p. into fresh mice and treated as above. After 3 transplant generations, high inocula of viable DTIC-treated cells were rejected by untreated syngeneic animals. Thereafter, antigenic sublines were maintained in mice immunosuppressed with CY (200 mg/kg/24 hr before tumor challenge) without further treatments.

Immune Lymphocytes. In order to obtain immune lymphocytes: (a) a single i.p. injection of viable 10 x 10^6 DTIC cells

---

1 Research was supported in part by Contract CT 78.01801—PFCCN from Consiglio Nazionale delle Ricerche, Rome, Italy.
2 To whom requests for reprints should be addressed, at National Cancer Institute, Building 31, Room 10A-22, Bethesda, Md. 20205.

Received March 19, 1980; accepted January 9, 1981.
was administered to CD2F mice; and (b) for the parent L1210 lines, 20 x 10⁶ X-ray-inactivated (5000-R Securix compact; 200 kV; 12 ma; 58.3 R/min) cells were injected i.p. into CD2F mice. The optimal immunizing schedule used here had been determined in preliminary studies.

Spleen cells from tumor-treated CD2F mice were collected 15 days after the immunizing inoculum and washed, and 20 x 10⁶ cells/mouse/0.4 ml i.v. were inoculated in recipient mice as reported previously (16).

Drugs. The citric salt of DTIC dissolved in chilled distilled water, BCNU dissolved in a few drops of ethyl alcohol and the volume adjusted with chilled 0.85% NaCl solution, and CY dissolved in the NaCl solution immediately before use were obtained from Dr. V. L. Narayanan, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

RESULTS

The enhanced resistance to the challenge of parental leukemia, exhibited by histocompatible animals immunized with living L1210/DTIC cells, relative to the resistance of animals immunized with inactivated L1210 parental cells, served as a basis for the therapeutic treatment of leukemic animals.

Direct stimulation of host reactivity by inoculation of viable L1210/DTIC cells 3 hr after the challenge with LH and LCr lines was essentially ineffective in increasing the survival time of leukemic mice (Table 1). There was an indication of therapeutic effect only with the lowest inoculum of LHa cells (10⁵) followed by treatment with LHa/DTIC cells. The rapidly lethal growth of the parental tumors apparently did not permit the animals to develop effective immune reactions prior to death. Antigenic stimulus with viable DTIC cells, given 24 or 48 hr after parental tumor challenge, was also ineffective. Also, the survival time of leukemic mice inoculated i.p. with either X-ray-inactivated LHa or LCr cells was not modified relative to that of untreated leukemic mice (Table 1).

Although L1210/DTIC cells did not provide a therapeutic effect on the majority of leukemic animals, some activity was elicited by these cells when accompanied by treatment with a chemotherapeutic agent. Mice inoculated with leukemia LHa were treated 3 hr later with LHa/DTIC cells followed by administration of BCNU (18 mg/kg i.p.) on Day +6 after leukemic challenge. With this regimen, the survival time was longer than that observed for mice treated with BCNU alone or with BCNU plus X-ray-inactivated LHa cells, and a number of animals were cured (Table 2). The enhancement of therapeutic response was less evident on treatment with LHa/DTIC cells plus BCNU (30 mg/kg).

A therapeutic effect in mice with LCr leukemia was obtained using a treatment protocol in which viable LCr/DTIC cells and adoptive transfer of preliminized lymphocytes were combined with BCNU treatment. The protection was relatively moderate, the highest incidence of cures occurring at the lowest inoculum level of leukemic cells (Table 3).

Therapeutic synergism was marked, using the protocols reported in Tables 4 and 5 in which chemotherapy preceded the transfer of lymphocytes immune to LCr/DTIC cells. In the first experiment, CY (120 mg/kg) was administered 24 hr after inoculation of LCr leukemia and followed 1 day later by adoptive transfer of normal lymphocytes or lymphocytes immune to either LCr or LCr/DTIC leukemic cells. There was little evidence of synergism on combination therapy with CY and lymphocytes from animals that had been immunized against LCr leukemia (Table 4).

CY treatment followed by inoculation of immune lymphocytes was also used in an effort to cure mice with advanced leukemia (27). CY was administered at a higher dosage (180 mg/kg) 5 days after the challenge with LCr leukemia, and this was followed, 24 hr later, by inoculation of lymphocytes immune to LCr/DTIC cells (Table 5). With this combined modality of therapy, most of the animals survived. In contrast, a lower number of animals survived following treatment with CY plus lymphocytes from animals immune to LCr leukemia. CY plus supplementary therapy with syngeneic lymphocytes from non-immunized animals was ineffective.

Long-term survivors, apparently tumor free, 60 days following the treatment modality presented in Table 4 (LCr on Day 0, CY on day +1, and spleen lymphocytes from CD2F mice immune to LCr/DTIC leukemia on Day +2), were rechallenged with 10³ LCr leukemia without any further treatment. Resistance to challenge with the parental tumor was evidenced by the surviving animals (Table 6).

DISCUSSION

The present study shows that immunogenic cells, obtained following drug treatment of murine leukemias, can be used for generating successful immunochemotherapy regimens against the parental tumor.

The drug used for increasing the immunogenicity of murine lymphomas was DTIC, an antineoplastic agent capable of mediating the appearance of novel transplantation antigens on tumor cells, without loss of the original TAA (1). Similar drug-induced antigenic changes have been described in teratoma cells (3) and normal lymphocytes (22) exposed to mutagenic compounds.

The observation that drug-altered leukemic cells retain TAA of the parental line provided a rational basis for immunotherapeutic application of these immunogenic cells. A similar approach has been used by other investigators with immunotherapy models in mice in which antigenic cross-reactivity was related to the presence of common Friend-Moloney-Rauscher group antigens (5, 7).

In the current study, drug-altered immunogenic lymphoma sublines were used for active immunization of leukemic mice and for the generation of immune lymphocytes used for adoptive transfer into syngeneic recipients.

No more than limited effects were observed when immunogenic leukemia cells were used for primary sensitization of lymphoma-bearing mice. Investigation of the growth kinetics of the LHa/DTIC subline in a previous study (24) showed that the tumor grows in the peritoneal cavity of the host during the first 4 days after challenge. Thereafter, the rejection process is readily detectable by the decline of both proliferation rate and number of tumor cells. Thus, it would appear that a definitive graft response is present in mice from Day +4 onward following challenge with the LHa/DTIC line.

The rapid growth of the moderately immunogenic parental LHa line, injected shortly before the i.p. transfer of drug-treated

* A. Nicolin, unpublished observations.
### Table 1

<table>
<thead>
<tr>
<th>No. of L1210 cells inoculated</th>
<th>Untreated</th>
<th>LHaxa</th>
<th>LHax/DTIC</th>
<th>LCrx</th>
<th>LCrx/DTIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
</tr>
<tr>
<td>LHax</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>13</td>
<td>11-16</td>
<td>12/12</td>
<td>16</td>
</tr>
<tr>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>11</td>
<td>9-14</td>
<td>12/12</td>
<td>12</td>
<td>9-15</td>
</tr>
<tr>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>10</td>
<td>8-10</td>
<td>12/12</td>
<td>10</td>
<td>9-12</td>
</tr>
<tr>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>7</td>
<td>6-9</td>
<td>12/12</td>
<td>8</td>
<td>7-9</td>
</tr>
</tbody>
</table>

LHaxa, X-ray-inactivated LHax cells; MST, median survival time; D/T, dead animals/total.

### Table 2

**Chemoimmunotherapy of mice bearing LHax leukemia with an inoculum of viable LHax/DTIC cells and BCNU**

<table>
<thead>
<tr>
<th>CD2F, mice challenged i.p. with graded doses of LHax leukemia or LCrx leukemia went untreated or were inoculated i.p. 3 hr later with 10&lt;sup&gt;7&lt;/sup&gt; viable LHax/DTIC or 10&lt;sup&gt;7&lt;/sup&gt; LCrx/DTIC cells, or 2 x 10&lt;sup&gt;7&lt;/sup&gt; X-ray-inactivated LHax or LCrx cells.</th>
<th>Untreated</th>
<th>LHax&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LHax/DTIC</th>
<th>BCNU&lt;sup&gt;b&lt;/sup&gt;</th>
<th>LHax + BCNU</th>
<th>LHax/DTIC + BCNU</th>
<th>BCNU&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LHax + BCNU</th>
<th>LHax/DTIC + BCNU</th>
<th>BCNU&lt;sup&gt;d&lt;/sup&gt;</th>
<th>LHax + BCNU</th>
<th>LHax/DTIC + BCNU</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
</tr>
<tr>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>19</td>
<td>13-27</td>
<td>9/12</td>
<td>24</td>
<td>10-32</td>
<td>6/12</td>
<td>55</td>
<td>1/12</td>
<td>16</td>
<td>15-24</td>
<td>10/12</td>
<td>17</td>
</tr>
<tr>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>13</td>
<td>11-17</td>
<td>12/12</td>
<td>18</td>
<td>14-21</td>
<td>10/12</td>
<td>18-53</td>
<td>6/12</td>
<td>17</td>
<td>14-23</td>
<td>12/12</td>
<td>17</td>
</tr>
<tr>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>10</td>
<td>9-13</td>
<td>12/12</td>
<td>13</td>
<td>10-18</td>
<td>12/12</td>
<td>17</td>
<td>13-43</td>
<td>8/12</td>
<td>14</td>
<td>11-20</td>
<td>12/12</td>
</tr>
<tr>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8</td>
<td>7-10</td>
<td>12/12</td>
<td>11</td>
<td>10-19</td>
<td>12/12</td>
<td>14</td>
<td>11-19</td>
<td>9/12</td>
<td>12</td>
<td>9-15</td>
<td>12/12</td>
</tr>
</tbody>
</table>

LHaxa, X-ray-inactivated LHax cells; MST, median survival time; D/T, dead animals/total.

### Table 3

**Chemoimmunotherapy of mice bearing LCrx with LCrx/DTIC cells, lymphocytes immune to LCrx/DTIC, and BCNU**

<table>
<thead>
<tr>
<th>Neither leukemic cells nor immune lymphocytes given separately increased the survival time of untreated leukemic mice or enhanced the antitumor activity of BCNU.</th>
<th>Untreated</th>
<th>LCrx&lt;sup&gt;e&lt;/sup&gt;</th>
<th>LCrx/DTIC&lt;sup&gt;f&lt;/sup&gt;</th>
<th>BCNU&lt;sup&gt;g&lt;/sup&gt;</th>
<th>LCrx + BCNU</th>
<th>LCrx/DTIC + BCNU</th>
<th>BCNU&lt;sup&gt;h&lt;/sup&gt;</th>
<th>LCrx + BCNU</th>
<th>LCrx/DTIC + BCNU</th>
<th>BCNU&lt;sup&gt;i&lt;/sup&gt;</th>
<th>LCrx + BCNU</th>
<th>LCrx/DTIC + BCNU</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
<td>MST</td>
<td>Range</td>
<td>D/T</td>
</tr>
<tr>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>12</td>
<td>11-14</td>
<td>12/12</td>
<td>16</td>
<td>13-18</td>
<td>10/12</td>
<td>13</td>
<td>11-16</td>
<td>12/12</td>
<td>21</td>
<td>18-39</td>
<td>9/12</td>
</tr>
<tr>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10</td>
<td>9-13</td>
<td>12/12</td>
<td>15</td>
<td>12-19</td>
<td>12/12</td>
<td>11</td>
<td>9-13</td>
<td>12/12</td>
<td>17</td>
<td>14-23</td>
<td>11/12</td>
</tr>
<tr>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>9</td>
<td>8-10</td>
<td>12/12</td>
<td>15</td>
<td>14-19</td>
<td>12/12</td>
<td>9</td>
<td>9-10</td>
<td>12/12</td>
<td>10</td>
<td>7-16</td>
<td>12/12</td>
</tr>
</tbody>
</table>

CD2F, mice challenged i.p. with graded doses of LCrx leukemia, went untreated or were inoculated i.p. 3 hr later with 10<sup>7</sup> viable LHax/DTIC or 10<sup>7</sup> LCrx/DTIC cells, or 2 x 10<sup>7</sup> X-ray-inactivated LHax or LCrx cells. Dosage, 18 mg/kg, i.p. on Day +6.

Since synergistic antitumor effects have been obtained by combining graft responses and chemotherapy in various animal models (12, 23), an immunochemotherapy protocol was adopted in an attempt to exploit the minimal effectiveness of the primary graft response against the LHax/DTIC line in leukemia-bearing hosts. BCNU (18 mg/kg) plus immunotherapy with LHax/DTIC cells resulted in significant enhancement of therapy (Table 2). The lack of significant therapeutic enhancement by the higher dose of the drug (i.e., 30 mg/kg) could be attributable to immunodepressive activity of this alkylating agent. Previous investigations have shown that chemotherapy plus adoptive transfer of immune syngeneic lymphocytes can afford marked antitumor protection against murine lymphomas (6, 7). With the aggressive L1210 Cr leukemia, the therapeutic enhancement was not as extensive when anti-LCr/DTIC immune lymphocytes were transferred into leukemic hosts, followed by BCNU chemotherapy (Table 3). However, more extensive therapeutic enhancement was obtained by combining active plus adoptive immunity (Table 3).
Chemotherapy and Immunotherapy in L1210 Leukemic Mice

Chemoimmunotherapy of mice bearing early LCr leukemia with CY and lymphocytes immune to LCr/DTIC

CD2F, animals, challenged i.p. with graded doses of LCr leukemia, were treated with CY, 120 mg/kg i.p., on Day +1. On Day +2, 2 x 10^7 CD2F, spleen cells from normal animals, from animals sensitized with 2 x 10^7 X-ray-inactivated leukemia cells i.p., or from animals sensitized with 10^7 L1210/DTIC leukemia cells i.p. were inoculated i.v. Adoptive transfer of either normal or immune lymphocytes in the absence of CY treatment did not increase the survival time of leukemic mice over untreated controls.

<table>
<thead>
<tr>
<th>No. of LCr cells inoculated i.p.</th>
<th>Untreated</th>
<th>CY (Day +1) alone</th>
<th>CY (Day +1) + adoptive transfer (Day +2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MST Range</td>
<td>MST Range</td>
<td>MST Range</td>
</tr>
<tr>
<td>10^3</td>
<td>12 11-14 8/8</td>
<td>17 13-24 7/8</td>
<td>16 15-21 6/8</td>
</tr>
<tr>
<td>10^4</td>
<td>10 9-13 8/8</td>
<td>15 11-19 8/8</td>
<td>14 12-17 8/8</td>
</tr>
<tr>
<td>10^5</td>
<td>8 7-9 8/8</td>
<td>10 9-14 8/8</td>
<td>9 8-13 8/8</td>
</tr>
<tr>
<td>10^6</td>
<td>7 7-8 8/8</td>
<td>10 8-11 8/8</td>
<td>9 6-11 8/8</td>
</tr>
</tbody>
</table>

* MST, median survival time; D/T, dead animals/total; LCr, spleen lymphocytes from CD2F, mice immune to LCr leukemia; LCr/DTIC, spleen lymphocytes from CD2F, mice immune to LCr/DTIC leukemia. N, spleen lymphocytes from normal CD2F, mice.

Marked improvement of the therapeutic efficacy resulted when leukemic mice were treated with CY followed by adoptive transfer of immune lymphocytes (Tables 4 and 5). This is in agreement with previous observations concerning the efficacy of immunotherapy protocols where CY treatment preceded the transfer of immunocompetent cells (26). It is suggested that conditioning of the host with CY administration may promote the settlement and proliferation of transferred lymphocytes, in addition to any reduction of the tumor burden. It is also possible that treatment with CY may inhibit suppressor cells present in lymphoma-bearing mice (9, 13).

The observation that it is possible to activate autologous lymphocytes in vitro (25) and the ability to grow cytotoxic lymphocytes in long-term culture (8) may contribute to furthering the current approaches. In addition, cloning procedures for long-term culture of cytotoxic lymphocytes (4) might result in a higher level of cell-mediated lytic activity for both L1210/DTIC and parental L1210 cells.

The existence, for the success of any immunological treatment, including that proposed here, of TAA on tumor cells should be stressed. Although the immunogenicity of tumor cells with low immunogenic TAA can be enhanced with the chemotherapeutic approaches used here, it is still necessary that a target to immune reactions be present originally on the tumor cells that are to be attacked. As yet, target TAA has not been definitely demonstrated in spontaneous tumors.

ACKNOWLEDGMENTS

The authors acknowledge the kind assistance of Dr. J. Mayo and C. Reeder, Mammalian Genetics and Animal Production Section, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, for breeding and providing the animals. The proficient help of L. Ricci and F. Sarra is gratefully acknowledged.

REFERENCES

Chemotherapy and Immunotherapy of L1210 Leukemic Mice with Antigenic Tumor Sublines

Angelo Nicolin, Gianfranco Canti, Ornella Marelli, et al.


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/4/1358

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/41/4/1358. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.