Differences in the Immunogenicity of Leukemia L1210 Sublines in DBA/2 Mice

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SUMMARY

After the i.p. inoculation of 10^3, 10^4, or 10^5 leukemia L1210 cells, the survival of DBA/2Ha-DD mice was longer than that of DBA/2J or DBA/2Cr mice. This difference was greatly reduced in mice that received total body X-radiation (250 R) the day prior to cell inoculation and in nonirradiated mice after the inoculation of 10^6 or 10^7 L1210 cells. DBA/2Ha-DD, DBA/2J, and DBA/2Cr mice survived longer after the inoculation of cells from an L1210 line resistant to methylglyoxal-bis(guanylhydrazone) than after inoculation of cells from the parent L1210 line. These differences were also reduced in preirradiated mice.

Treatments with low doses of arabinosylcytosine were more effective against the L1210 cells resistant to methylglyoxal-bis(guanylhydrazone) than against the parent line in both DBA/2Ha-DD and DBA/2J mice; similar differences in therapeutic response were seen in DBA/2Ha-DD mice treated with 4,4'-diacetyldiphenylurea-bis(guanylhydrazone), 2-chloro-4',4''-di-2-imidazolin-2-ylterephthalanilide, or carzinostatin but were not seen in those treated with methotrexate. In DBA/2Ha-DD mice, arabinosylcytosine was also more effective against an L1210 line resistant to 4,4'-diacetyldiphenylurea-bis(guanylhydrazone) than against the parent line. All of these therapeutic differences were greatly reduced or abolished in preirradiated mice.

On the basis of transplantation tests in DBA/2Ha-DD and DBA/2J mice, a line of L1210 grown in culture for several months appeared to be more immunogenic than were any of the in vivo lines of L1210 examined.

INTRODUCTION

L1210 is considered to be a DBA/2 strain-specific leukemia, because it grows only in the DBA/2 mouse and in F_1 hybrids and backcrosses derived from it. In DBA/2 mice, the inoculation of 10 to 100 L1210 cells or less leads to progressive leukemic growth and eventually results in the death of all the animals affected. Notwithstanding its strict strain specificity, L1210 has been found to be immunogenic in BALB/c X DBA/2 F_1 hybrid (CDBA) mice (2), in C57BL/6 X DBA/2 F_1 hybrid (BDF_1) mice (14), in backcrossed [(DBA X C3H) F_1 X DBA] mice (13) and, more recently, in 3 sublines of DBA/2 mice (8). In the backcrossed mice, the host response elicited by the inoculation of X-irradiated L1210 cells was very weak and could be demonstrated only in mice challenged with 10 L1210 cells or less (13). In the DBA/2 mouse sublines, a host response became effective, even in animals given i.p. inoculations of 10^6 L1210 cells, when the growth of the leukemia was selectively impaired by certain chemotherapeutic treatments (7, 8). Although the immunological nature of this host response was indicated by the data (7), its detail mechanisms have not yet been clarified.

In initial experiments, a subline of L1210 resistant to CH_3-G^2 (L1210/CH_3-G) was found to be more sensitive than was L1210 to ara-C in nonirradiated but not in preirradiated DBA/2Ha-DD mice (8). This observation suggested that the differences in therapeutic effects noted in nonirradiated animals were due to differences in the host response elicited by the leukemias and thus implied the possibility that the immunogenicity of L1210/CH_3-G was greater than that of L1210 in the DBA/2 mouse used.

This report presents evidence that the immunogenicity of L1210/CH_3-G is greater than that of L1210 in 3 DBA/2 mouse sublines and that this greater immunogenicity is reflected by a greater sensitivity to the chemotherapeutic effects of ara-C and 3 other drugs. A subline of L1210 resistant to DDUG (L1210/DDUG) also appeared to be more immunogenic than L1210. A subline of L1210 grown in vitro was more immunogenic than were any of the in vivo lines tested. Some of these results have been reported previously in preliminary communications (3, 8, 9).

MATERIALS AND METHODS

The L1210 used in this study was obtained in 1957 from Dr. A. Goldin, National Cancer Institute, and thereafter was transferred every 6 to 7 days in female DBA/2Ha-DD mice by i.p. inoculation of 1 X 10^6 ascites cells. Sublines L1210/CH_3-G (6) and L1210/DDUG (5) were developed in this laboratory in 1966, and thereafter were transferred in a manner similarly to that used for L1210, except that the host mice were treated i.p. once daily for 4 to 6 days with CH_3-G, 50 mg/kg, and DDUG, 25 to 30 mg/kg, respectively.

The female DBA/2Ha-DD and DBA/2Cr mice were obtained from the breeding colony of this Institute; the DBA/2J mice were purchased from The Jackson Laboratory, Bar Harbor.

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The detailed history of the DBA/2Ha-DD mouse has been reported recently (7, 11). All the mice were used when 6 to 8 weeks old. The existence of non-H-2-related immunogenetic differences among the 3 mouse sublines used was suggested by the results of reciprocal skin-grafting tests (7) and by differences in chemotherapeutic response of L1210 (7, 8).

ara-C was obtained from Dr. G. Fonken (The Upjohn Co., Kalamazoo, Mich.); both CH3-G and NSC 38280 were obtained from Dr. H. Wood (Cancer Chemotherapy National Service Center, Bethesda, Md.); carzinostatin was obtained from Dr. J. Lein (Bristol Laboratories); DDUG was obtained from Professor A. Marxer (Ciba Research Laboratories, Basel, Switzerland). The drugs were dissolved in distilled water immediately before use and were injected i.p. once daily for 6 consecutive days, starting 1 day after the animals received i.p. inoculations of 10^6 leukemic cells. Mice surviving beyond the 50th day after implantation were considered cured and were then found to be resistant to a challenge with 10^3 live leukemic cells.

Total-body irradiation was administered by a General-Electric Model 250 Maxitron machine. The dose was calculated in roentgens, in air, and was given at 250 kVp and 30 ma. A 0.5-mm copper, 1.0-mm aluminum filter was used. Mice were confined in polyethylene plastic containers with adequate air supply and were placed at a skin target distance of 30 cm.

Throughout this study, differences in survival between nonirradiated and irradiated mice were taken as a measure of the immunogenicity of the leukemic cells inoculated or of the immunological reaction of different hosts. In each case, mice without leukemia were also treated with drugs and/or irradiation to exclude the possibility that toxicity complicated the interpretation of the data.

RESULTS

The survival of mice after the inoculation of L1210 or L1210/CH3-G cells was compared in 3 DBA/2 mouse sublines (Chart 1). After the inoculation of 10^5 L1210 cells or less, DBA/2Ha-DD mice survived longer than DBA/2J or DBA/2Cr mice. After the inoculation of 10^6 cells or more, no difference in survival of mice in these groups was noted. In each mouse subline, survival was longer after the inoculation of L1210/CH3-G than after inoculation of L1210. This difference was statistically significant with each cell inoculum in DBA/2Cr mice and with inocula of 10^5 cells or less in DBA/2Ha-DD and DBA/2J mice. Also, with L1210/CH3-G, DBA/2Ha-DD mice survived longer than DBA/2J or DBA/2Cr mice after the inoculation of 10^6 cells or less. No significant difference in survival was observed in preirradiated mice, regardless of the mouse subline or leukemia tested or of the number of cells inoculated.

Data reported previously (8, 11) indicated that the chemotherapeutic effects of DDUG and ara-C against L1210 were greater in DBA/2Ha-DD than in DBA/2J mice. These effects were reduced in preirradiated animals, and the differences between mouse sublines were abolished. In those studies, the differences in host response between DBA/2Ha-DD and DBA/2J mice were manifested against the same L1210 line. In this study, the opportunity was provided to compare chemotherapeutic effects under conditions in which differences in host response were related not only to differences in reactivity of the DBA/2 mouse subline used but also to differences in immunogeneticity of the L1210 line. This comparison was carried out with ara-C and 4 other drugs.

The therapeutic effects of relatively low doses of ara-C in nonirradiated and preirradiated DBA/2Ha-DD and DBA/2J mice bearing L1210 or L1210/CH3-G are shown in Chart 2. In DBA/2Ha-DD mice, ara-C was much more effective against L1210/CH3-G than against L1210, both in prolonging the survival of those mice which eventually died and in causing 50-day cures. Similar differences in therapeutic response were observed in DBA/2J mice. On comparison of the data shown in the upper and lower parts of Chart 2, it is apparent that the therapeutic effects against either leukemia line were greater in DBA/2Ha-DD than in DBA/2J mice and that all of the differences observed were minimized or abolished in animals that had been irradiated the day before they were given inoculations of leukemia cells. The survival of irradiated and drug-treated mice without leukemia indicated that toxicity was not a complicating factor in the interpretation of the results with leukemic mice (data not shown).

The results summarized in Table 1 indicate the greater therapeutic effects of 3 drugs, unrelated to ara-C, against L1210/CH3-G. In each case, the differences in therapeutic response were abolished by total-body X-radiation of the host (250 R), given 1 day prior to implantation (data not shown).
Treatments were given i.p. once daily for 6 consecutive days starting on the day prior to inoculation of the leukemic cells. For each drug, one-half of the dose shown in Table 1 was also used; the results are not shown because they did not add to or alter the meaning of the data presented. However, it should be specifically mentioned that, at the dose of 0.75 mg/kg/day, MTX did not exert greater effects against L1210/CH3-G than against L1210.

It was of interest to see whether a greater host response could be elicited by a subline of L1210 other than L1210/CH3-G. The comparative therapeutic effects of ara-C against L1210 and L1210/DDUG in DBA/2Ha-DD mice are shown in Chart 3. ara-C was more effective against L1210/DDUG than it was against L1210. No such differences were seen in mice given total-body X-radiation (250 R) the day prior to inoculation of the leukemic cells.

The possibility should be considered that the greater immunogenicity of L1210 sublines that are resistant to drugs with immunosuppressive action is related to a reduced immunoselection resulting from repeated treatments with increasing doses of the selecting drug during the development of resistance. The prolongation of skin graft survival by DDUG (7) and particularly by CH3-G (4) has been reported previously. It was of interest, therefore, to test the immunogenicity of a line of L1210 which had been transmitted in culture for a known period of time in the absence of any immunoselection. Line L1210-G, originated from Roswell Park Memorial Institute L1210 cell line 3116, which had been maintained in suspension cultures for 15 months by Dr. G. Grindey of this Department,3 was used as an example. As shown in Table 2, in DBA/2Ha-DD mice the inoculation of a minimum of 106 L1210-G cells was required in order to ensure progressive leukemia growth leading to death in one-half of the animals, whereas the inoculation of 107 cells was necessary to cause death in all of the mice. In preirradiated animals, however, death followed the inoculation of 105 cells or more. L1210-G appeared to be less immunogenic in DBA/2J mice than in DBA/2Ha-DD mice, since all the DBA/2J mice died after the inoculation of 105 cells or more. In comparing the survival data in Table 2 with those in Chart 1, however, it is apparent that L1210-G cells were considerably more immunogenic than L1210 or L1210/CH3-G cells in both DBA/2Ha-DD and DBA/2J mice. Also, L1210-G and L1210 have cross-reactive antigenic properties. In fact, after the inoculation of 104 to 105 cells, L1210-G did not grow in 15 preirradiated DBA/2Ha-DD mice that had been previously cured of L1210 by chemotherapeutic means (data not shown), in contrast with its growth in preirradiated mice which had not been exposed to L1210 at any time prior to irradiation.

**DISCUSSION**

On the basis of transplantation experiments performed in nonirradiated and preirradiated mice, it was found that the host defenses against L1210 are more effective in DBA/2Ha-DD than in DBA/2J or DBA/2Cr mice and also that they are more effective against L1210/CH3-G than against L1210 in each of the 3 DBA/2 mouse sublines studied. Consistent with the greater effectiveness of the defenses of the host against the resistant leukemic cells, treatments with low doses of ara-C and 3 other drugs were more effective against L1210/CH3-G than against L1210. The differences in therapeutic response were reduced or abolished in mice given total body X-radiation (250 R) on the day prior to inoculation of the leukemic cells and were also slightly reduced after treatments with ara-C at the highest dose used.

The differences in survival among comparable groups observed in this study can be attributed to differences in the response of host defenses to leukemic cells with different immunogenicity or to differences in the sensitivity of the leukemic cells to the response of the host. The argument in favor of this idea rests essentially upon the fact that these differences were not seen in preirradiated mice, namely under conditions in which the defenses of the host were greatly impaired. Recently, by means of a paired-label radioantibody technique with IgG 201I as used for L1210/CH3-G cells, it was found that L1210/CH3-G cells have more antigen than L1210 cells and, possibly, have antigens not expressed on the parent leukemia cells, as reflected by increased specific antibody-binding capacity in vitro (3). Thus it would seem unlikely that changes in cell membrane not related to antigenic characteristics are responsible for a greater nonspecific sensitivity of the drug-resistant cells to the defenses of the host in vivo.

The likelihood that L1210/CH3-G cells also express antigens different from those of L1210 would be supported but not proven by the observation that, in this study, the resistant cells were more immunogenic than were cells from the parent line

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in DBA/2J and DBA/2Cr mice also (those DBA/2 mouse sublines which react the least to L1210).

The possibility that differences in the therapeutic effects of ara-C were related to differences in pharmacodynamics between DBA/2Ha-DD and DBA/2J mice is negated by the fact that no such differences were noted in preirradiated animals. The alternative possibility that the effects of ara-C were reduced in preirradiated mice as a result of an increased availability of reversing metabolites originating from X-ray-damaged tissues seems very unlikely. In fact, results similar to those with ara-C were also obtained with DDUG, NSC 38280, and carzinostatin, and it would seem improbable that the action of each of these different drugs is reversed by products from damaged tissues. Moreover, without chemotherapy, the differences observed in transplantation tests also were abolished in preirradiated animals. This finding also excluded the possibility that intrinsic differences in cell kinetics between sensitive and resistant lines were responsible for the differences seen.

The results described in this report further support the concept, discussed previously (7, 8, 10, 11), that selective treatments with antileukemic drugs may exert therapeutic effects against weakly immunogenic and/or rapidly proliferating transplantable leukemias in cooperation with the immunological responses directed against the leukemic cells, responses that are relatively inefficient per se, and thus may pass unnoticed in the absence of chemotherapy. In the case of antileukemic drugs that also have immunosuppressive action,

Table 1

Comparative response of L1210 and L1210/CH3-G to chemotherapeutic agents in DBA/2Ha-DD mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>L1210 Average survival (days)</th>
<th>L1210/CH3 Average survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.1</td>
<td>7.7</td>
</tr>
<tr>
<td>DDUG 15</td>
<td>18.2</td>
<td>30.9</td>
</tr>
<tr>
<td>NSC 38280 18</td>
<td>30.8</td>
<td>11.0</td>
</tr>
<tr>
<td>Carzinostatin</td>
<td>15.6</td>
<td>25.5</td>
</tr>
<tr>
<td>MTX 1.5</td>
<td>15.2</td>
<td>11.7</td>
</tr>
</tbody>
</table>

* Treatment was given once daily starting the day after the i.p. inoculation of 10⁶ cells. Effects of representative doses only are shown to illustrate the differences of response between the 2 leukemia sublines.

* Survival of only those mice that died on or before Day 50.

Table 2

Survival of DBA/2Ha-DD and DBA/2J mice after the inoculation of different numbers of cultured L1210 cells

The number of cells indicated were inoculated i.p.; 250 R total-body radiation were given the day prior to tumor inoculation as indicated. Average survival values include only mice that died within 50 days after inoculation of the leukemic cells.

<table>
<thead>
<tr>
<th>DBA/2Ha-DD mice</th>
<th>DBA/2J mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>No X-ray</td>
<td>X-ray</td>
</tr>
<tr>
<td>No. of cells inoculated</td>
<td>Average survival (days)</td>
</tr>
<tr>
<td>10³</td>
<td>9/9</td>
</tr>
<tr>
<td>10⁴</td>
<td>9/9</td>
</tr>
<tr>
<td>10³</td>
<td>9/9</td>
</tr>
<tr>
<td>10⁴</td>
<td>19.8</td>
</tr>
<tr>
<td>10⁵</td>
<td>10.2</td>
</tr>
</tbody>
</table>

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such cooperative therapeutic effects against L1210 may be reduced at high drug dosages, as is possibly indicated by the reduced effectiveness of ara-C against L1210/CH₃-G at the 10-mg/kg dose (see Charts 2 and 3). The fact that MTX was not more effective against L1210/CH₃-G than against L1210 may also have a similar basis.

The results of this study also indicate that, at least in the case of the L1210 lines and the DBA/2 mouse sublines tested, the therapeutic effectiveness of certain drugs can be altered, depending upon differences in the response of the host, regardless of whether these differences reflect the reactivity of different hosts to the same leukemic cell line or the reactivity of the same hosts to different leukemic cell lines. The implications of these results in experimental chemotherapy are obvious, not only in evaluating the effects of new drugs but also in determining whether an increased therapeutic response against a resistant tumor is related to increased cellular sensitivity to the drug, namely "collateral sensitivity," or to an increased immunogenicity of the resistant cells.

At this time the question can be posed only as to whether the increased immunogenicity of L1210/CH₃-G cells in the DBA/2 mouse sublines studied is specifically and directly related to the metabolic changes occurring in these cells during the development of resistance. The fact that L1210/CH₃-G retained both its characteristic resistance and its increased immunogenicity in DBA/2Ha-DD mice after 185 transplant generations in these mice without CH₃-G maintenance treatment (unpublished results) suggests that these changes are genetically determined and that the greater immunogenicity of the resistant cell population is relatively stable in the face of immunoselection in the absence of treatments with CH₃-G, a drug with immunosuppressive action (4). The finding that L1210/DDUG also was more immunogenic than L1210 in DBA/2Ha-DD and the recent observations that other L1210-resistant sublines may be more immunogenic than parent L1210 in F₁ hybrids of DBA/2 (1, 12, 15, 16) make it unlikely that the change in L1210/CH₃-G cells is specifically and directly related to the development of resistance to CH₃-G.

The possibility that increased antigenic expression in resistant L1210 cell populations is related to the immunosuppressive effects of the drugs selecting for resistance, effects which might result in a reduction of immunoselection, was supported by the fact that a cultured L1210 cell line, sheltered from immunoselection for several months, was found to be more immunogenic than was L1210 in both DBA/2Ha-DD and DBA/2J mice. However, resistance to CH₃-G can be developed very rapidly, within 1 to 3 transplant generations (6). Thus a decrease in immunoselection possibly resulting from CH₃-G treatment may not persist long enough to be responsible for the greater antigenicity of L1210/CH₃-G.

In conclusion, the initial observation that L1210/CH₃-G is more immunogenic than L1210 in DBA/2Ha-DD mice (8) has been substantiated in this study; this observation is consistent with analogous findings subsequently reported by others with L1210 sublines resistant to different drugs (1, 12, 15, 16). The overall evidence obtained in vitro study of the antigenicity of L1210 and L1210/CH₃-G (3), indicates that L1210 cells resistant to certain chemotherapeutic agents are more immunogenic than are the parent cells.

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