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# Combined Effects of Antineoplastic Agents and Anti-Lymphoma Allograft Reactions*

CARLO RICCARDI,† ANNA BARTOCCI,† PAOLO PUCCETTI,† *FEDERICO SPREAFICO*,‡ ENZO BONMASSAR† and ABRAHAM GOLDIN§

†*Institute of Pharmacology, University of Perugia, Via del Giochetto, Perugia* 06100 *Italy*
‡*Mario Negri Institute Via Eritrea, Milano* 20100 *Italy*
§*National Cancer Institute, National Institutes of Health, Bethesda, Maryland* 20014 *U.S.A.*

**Abstract**—*Combined effects of chemotherapy and anti-lymphoma allograft responses were studied in mice inoculated intraperitoneally (i.p.) with leukemia cells incompatible for multiple minor histocompatibility loci* (MMHL) *and treated with graded doses of* 1,3-*bis*(2-*chloroethyl*)-1-*nitrosourea* (*BCNU*), *vincristine* (*VCR*), *cyclophosphamide* (Cy), 5-(3,3-*dimethly*-1-*triazeno*)-*imidazole*-4-*carboxamide* (*DTIC*) *or hexamethyl-melamine* (*HMM*). *The results showed that:* (*a*) *no appreciable difference in survival times was found, as a rule, in leukemic compatible or allogeneic mice not subjected to drug treatment;* (*b*) *both BCNU and Cy markedly prolonged the survival times of compatible leukemic mice, but only treatment with BCNU resulted in chemo-immune collaborative activity when the drug was administered to allogeneic hosts;* (*c*) *DTIC was moderately active in compatible mice and no additive or synergistic effects were found in allogeneic hosts;* (*d*) *VCR, little or no effect in prolonging the survival times of leukemic compatible mice, proved to be highly efficient when given to allogeneic recipients;* (*e*) *HMM was totally ineffective in prolonging the survival time of either compatible or allogeneic mice. These studies evidenced that no obvious relationship could be found between the anti-leukemic activity of different drugs and their antineoplastic effectiveness when combined with antitumor immune responses.*

## INTRODUCTION

One of the most challenging problems of cancer chemotherapy is represented by the limited selectivity of oncotherapeutic agents against neoplastic cells. Toxicity affecting the immune system appears to be a matter of considerable concern since it is largely assumed that host antitumor responses play a crucial role in controlling tumor growth [1–3]. Moreover, additive or synergistic effects between immunotherapy and chemotherapy have been described in the literature [4–6]. Extensive studies performed by Mihich [7] showed that complete cure of mice bearing experimental tumors could be obtained when chemotherapeutic drugs were given to animals capable of mounting host-anti-graft responses. This was confirmed in a host–tumor system in which congenic-resistant mice were inoculated with $H-1$ incompatible lymphoma cells and treated with BCNU [8].

The present investigation was carried out using three experimental models in which two murine lymphoma lines were used and relied upon the general assumption that reactivity to alloantigens is related to reactivity against tumor-associated antigens [1, 9]. Therefore, different degrees of anti-tumor allograft responses were obtained by means of an appropriate design of genetic distance between host and tumor. The results of these studies showed that marked synergism between chemotherapy and allograft responses could be obtained with antitumor agents either active (BCNU) or almost inactive (VCR) in histocompatible leukemic recipients. However, the efficacy of other compounds, highly (Cy) or moderately (DTIC) active in compatible mice, did not change substantially in allogeneic hosts.

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## MATERIALS AND METHODS

### *Mice*

Inbred B10.D2 Cr($H-2^d$), BALB/c Cr($H-2^d$) and hybrid (BALB/c Cr × DBA/2 Cr)$F_1$(CD2$F_1$, $H-2^d/H-2^d$), (C57B1/6 Cr × DBA/2 Cr)$F_1$ (BD2$F_1$, $H-2^b/H-2^d$) mice of both sexes, 5–6 months old, were obtained from the Mammalian Genetics and Animal Production Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

### *Tumors*

L1210 Cr, an ascites leukemia of DBA/2 origin [10] was carried in DBA/2 or CD2$F_1$ mice, and LSTRA, an ascitic lymphoma induced by Moloney leukemia virus in BALB/c mice [11], was carried in female mice of the strain of origin. The tumors were maintained by weekly passages of neoplastic cells suspended in medium 199 by i.p. route. Mortality was recorded for at least 60 days after tumor challenge and the presence of ascites and/or generalized lymphoma was confirmed at autopsy.

### *Drugs*

BCNU, Cy, VCR, DTIC and HMM were kindly supplied by Dr. H. B. Wood, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland. BCNU, Cy and VCR were dissolved in 0.85% NaCl solution immediately before use; DTIC was dissolved in 1% citric acid solution; HMM was suspended in 0.85% NaCl solution containing 1% Tween 80. All drugs were injected i.p. in volumes of 0.1ml/10g of body weight.

### *Irradiation*

Mice were exposed to whole-body irradiation (400r) 4–6 hr before tumor cell transplantation in a $^{60}$Co-irradiator (Hot Spot MKIV, Harwell, England) delivering $\gamma$-rays at the rate of 1200 rev/min.

### *Statistical analysis*

The mortality of mice was analyzed according to Mann–Whitney U-Test and $P$ levels $<0.05$ were considered within the limits of significance. In general a difference in the median survival time (MST) of 5 days gave $P<0.05$, and therefore was considered statistically significant.

## RESULTS

The genetic relationship between host and tumor and the strength of anti-lymphoma allograft reaction evidenced by tumor titration are illustrated in Table 1. Different doses of L1210 or LSTRA lymphoma cells were injected into compatible or MMHL-incompatible hosts. Mortality data did not clearly reveal the existence of allograft responses being mounted by BALB/c mice inoculated with L1210 leukemia or BD2$F_1$ hosts challenged with LSTRA lymphoma. Only B10.D2 mice survived longer than compatible CD2$F_1$ hosts, following challenge with $10^6$ cells of LSTRA lymphoma. The occurrence, however, of an immune response being elicited by MMHL was confirmed by presensitization experiments in which BD2$F_1$ mice could resist LSTRA challenge following previous injection of $\gamma$-irradiated tumor cells (Table 2). Cyclophosphamide, a well known immunodepressive agent, could remove resistance when administered a few hours after tumor vaccine.

Graded doses of BCNU were injected into mice, 3 days after challenge with $10^6$ cells of compatible or MMHL-incompatible L1210 or LSTRA lymphomas. The optimal dose against L1210 leukemia in compatible CD2$F_1$ mice was 30 mg/kg, which produced marked increase of the median survival time (Fig. 1A). However, at this dose the percentage of long-term survivors did not exceed 10%. At 50 mg/kg the drug was highly toxic and the majority of mice died without evidence of leukemia growth. The MST was lower than that obtained with 30 mg/kg, although a higher percentage of long-term survivors was found. In MMHL-incompatible BALB/c mice BCNU was extremely active for a wide range of the doses used (i.e., from 3.88 to 30 mg/kg), and the majority of drug-treated leukemic hosts survived more than 60 days. For example, at the dose of 3.88 mg/kg BCNU treatment increased the MST of compatible CD2$F_1$ mice by only 33.3% over untreated controls, whereas all allogeneic BALB/c mice subjected to the same treatment survived beyond the 60-day observation period. Again at 50 mg/kg BCNU was toxic and more than 50% of BALB/c mice died without detectable leukemia.

Graded doses of BCNU were administered to CD2$F_1$ mice bearing LSTRA lymphoma cells (Fig. 1B). At 50 mg/kg the nitrosourea did not induce toxic deaths in more than 50% of the mice. It follows that the MST was not

*Table 1. Mortality of compatible or MMHL-incompatible mice inoculated with graded doses of L1210 or LSTRA lymphomas*

| Host | Tumor | Tumor/host incompatibility | Number of tumor cells i.p. $10^6$ MST* | $10^6$ D/T† | $10^4$ MST | $10^4$ D/T | $10^2$ MST | $10^2$ D/T |
|---|---|---|---|---|---|---|---|---|
| $CD2F_1$ | L1210 | — | 7 | 8/8 | 10 | 8/8 | 11.5 | 8/8 |
| BALB/c | L1210 | $H-1$, $H-3$, $(H-4)$,‡ $H-7$ $H-8$, $H-9$, $H-13$ | 8 | 8/8 | 11.5 | 8/8 | 14 | 5/8 |
| $CD2F_1$ | LSTRA | — | 8 | 8/8 | 11 | 8/8 | 14 | 7/7 |
| $BD2F_1$ | LSTRA | $H-1$, $H-3$, $H-4$,‡ $H-7$ $H-8$, $H-9$, $H-13$ | 8 | 8/8 | 12.5 | 6/6 | 14 | 6/8 |
| B10.D2 | | $H-1$, $H-3$, $H-4$,‡ $H-7$ $H-8$, $H-9$, $H-13$ | 14.5 | 4/6 | NT§ | | NT | |

*MST, median survival time.
†D/T, dead over total mice injected.
‡*The* $H-4$ allele is unknown in BALB/c. therefore, incompatibility at the $H-4$ locus is uncertain.
§NT, not treated.

*Table 2. Effect of presensitization on mortality of compatible $CD2F_1$ or MMHL-imcompatible $BD2F_1$ mice challenged with $10^6$ LSTRA lymphoma cells*

| Host | Presensitization* | Treatment | MST† | D/T‡ |
|---|---|---|---|---|
| $CD2F_1$ | − | None | 8 | 6/6 |
| $BD2F_1$ | − | None | 10 | 6/6 |
| $CD2F_1$ | + | None | 8 | 6/6 |
| $BD2F_1$ | + | None | — | 0/6 |
| $BD2F_1$ | + | Cy§ | 15 | 4/6 |

*Mice were injected i.p. with $25 \times 10^6$ $\gamma$-irradiated (5000 rad) LSTRA lymphoma cells 14 days prior to tumor challenge.
†MST, median survival time.
‡D/T, dead over total mice tested.
§Cyclophosphamide was given i.p. 200 mg/kg 5 hr after tumor cell vaccine.

reached at this dose level. Marked synergistic effects with allograft reaction were detected when BCNU (6.48–50 mg/kg) was used in allogeneic $BD2F_1$ recipients (Fig. 1B). Chemo-immune collaborative activity was also evidenced when 6.48–30 mg/kg of BCNU were used in B10.D2 hosts inoculated with LSTRA cells. At 50 mg/kg of the drug, the majority of allogeneic B10.D2 mice died without gross evidence of leukemia growth (Fig. 1C).

Further experiments were conducted with DTIC, using the same animal-tumor models described for BCNU. The results illustrated in Fig. 2 show that DTIC was moderately active in $CD2F_1$ mice carrying the compatible L1210 leukemia, or in BALB/c recipients carrying syngeneic LSTRA tumor. In both cases treatment with 320–400 mg/kg of DTIC afforded an increase of the MST of approximately 60% over that of untreated controls. When DTIC was given to MMHL-incompatible BALB/c or $BD2F_1$ mice carrying L1210 or LSTRA lymphoma respectively, marginal or no increase in the therapeutic efficiency of the drug was detected, as evidenced by the limited survival times and the lack of long-term survivors (Figs. 2A and 2B). Treatment with DTIC was slightly more effective in allogeneic B10.D2 than in syngeneic BALB/c mice bearing LSTRA lymphoma cells (Fig. 2C), when used at the dose of 163 or 204 mg/kg i.p. However, the increase in the MST of treated B10.D2 hosts was minimal if compared with

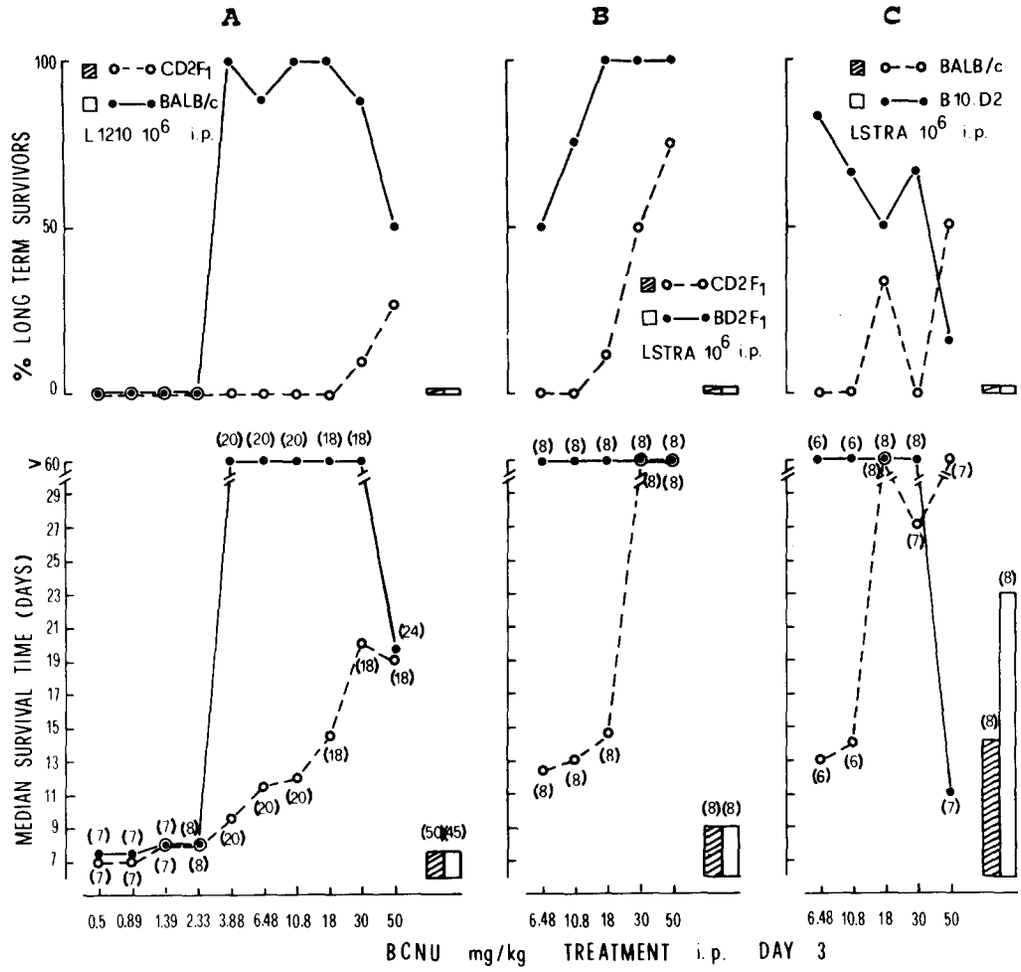


*Fig. 1. Antileukemic effect of graded doses of BCNU, in histocompatible $CD2F_1$ or incompatible BALB/c mice injected with L 1210 $10^6$ i.p. (A); in histocompatible $CD2F_1$ or incompatible $BD2F_1$ mice injected with LSTRA $10^6$ i.p. (B); in histocompatible BALB/c or incompatible B10.D2 mice injected with LSTRA $10^6$ i.p. (C). In parenthesis is the number of observations.*

the survival time of untreated controls. Moreover, no difference between DTIC-treated (163 mg/kg) or untreated B10.D2 mice was found if the percentages of long-term survivors are considered.

Further experiments were conducted to study the antitumor effects of DTIC in compatible or MMHL-incompatible mice when the drug was administered daily for 7 days after challenge. As shown in Table 3, three different doses of DTIC (100, 60 and 36 mg/kg i.p.) were injected in histocompatible or histoincompatible mice previously inoculated i.p. with $10^6$ L1210 lymphoma cells. Again, DTIC was almost completely inactive against L1210 leukemia cells, and no significant difference in survival times was detected between compatible $CD2F_1$ or MMHL-incompatible BALB/c hosts. The antitumor effect of graded doses of Cy in compatible or allogeneic mice was studied in the three host–tumor systems previously described. When Cy was tested in the L1210 model, in most cases comparable survival times were found between compatible $CD2F_1$ mice and allogeneic BALB/c hosts inoculated with graded doses of the antineoplastic agent (Fig. 3A). However, at the dose of 108 and 180 mg/kg the antitumor effect of Cy was more pronounced in allogeneic than compatible mice. In addition, higher efficiency of Cy in allogeneic recipients with respect to that detectable in compatible hosts, was evidenced in the LSTRA lymphoma system, either in incompatible $BD2F_1$ mice (Fig. 3B) treated with 180 and 300 mg/kg, or in B10.D2 recipients inoculated with 38.8, 64.8 and 108 mg/kg of Cy. Leukemic B10.D2 mice treated with higher

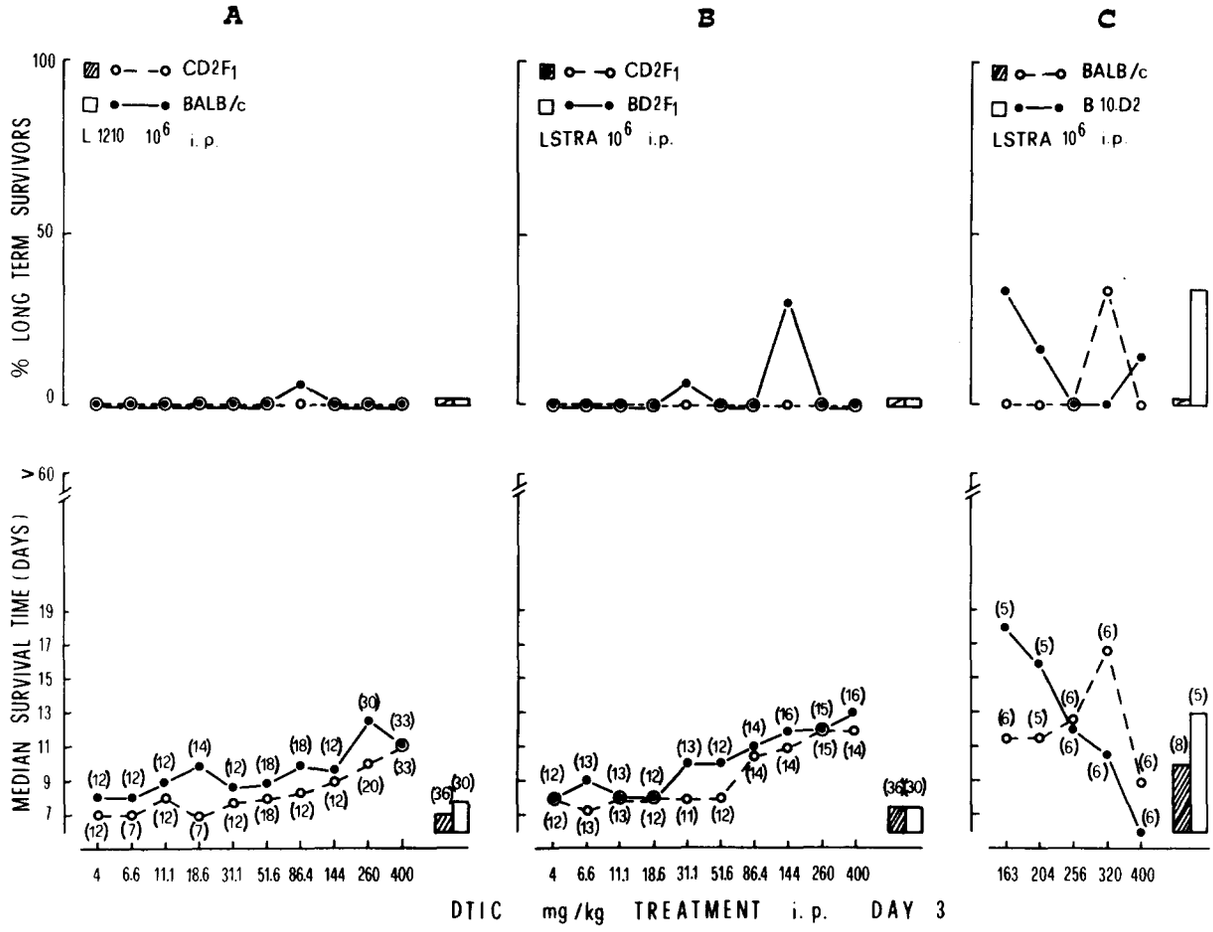


*Fig.* 2. *Antileukemic effect of graded doses of DTIC, in histocompatible $CD2F_1$ or incompatible BALB/c mice injected with L1210 $10^6$ i.p. (A); in histocompatible $CD2F_1$ or incompatible $BD2F_1$ mice injected with LSTRA $10^6$ i.p. (B); in histocompatible BALB/c or incompatible B10.D2 mice injected with LSTRA $10^6$ i.p. (C).*

*Table* 3. *Effect of multiple treatment with DTIC on L1210 leukemia in compatible $CD2F_1$ or MMHL-incompatible BALB/c mice*

| Host | Tumor i.p. | Treatment with DTIC dose† | day | MST* | D/T‡ |
|---|---|---|---|---|---|
| $CD2F_1$ | $10^6$ | — | — | 7 | 10/10 |
| $CD2F_1$ | $10^6$ | 100 | 1–7§ | 9 | 10/10 |
| $CD2F_1$ | $10^6$ | 60 | 1–7 | 9 | 10/10 |
| $CD2F_1$ | $10^6$ | 36 | 1–7 | 8 | 10/10 |
| BALB/c | $10^6$ | — | — | 7 | 10/10 |
| BALB/c | $10^6$ | 100 | 1–7 | 11 | 10/10 |
| BALB/c | $10^6$ | 60 | 1–7 | 10 | 10/10 |
| BALB/c | $10^6$ | 36 | 1–7 | 11 | 10/10 |

*MST, median survival time.
†mg/kg per mouse.
‡D/T, dead over total mice injected.
§Daily, from day 1–7.

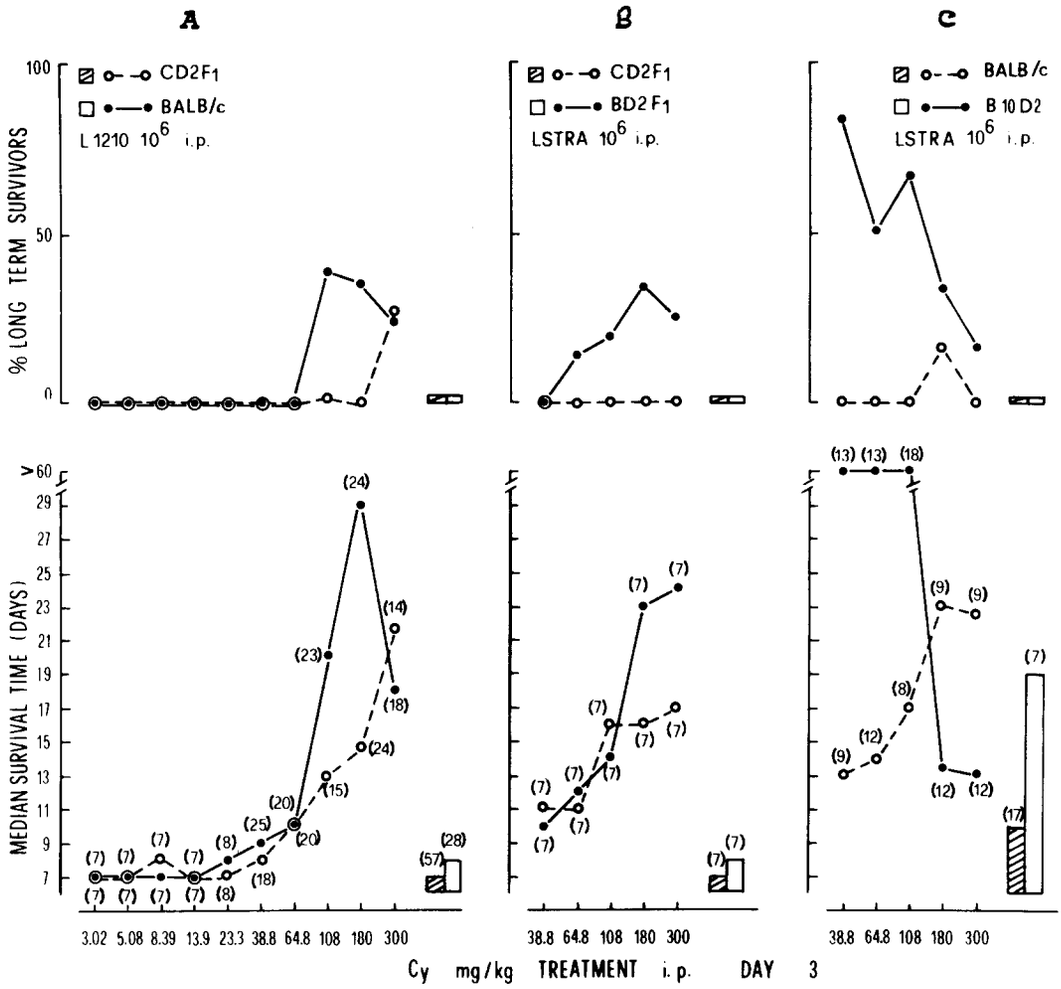


*Fig. 3. Antileukemic effect of graded doses of Cy, in histocompatible $CD2F_1$ or incompatible BALB/c mice injected with L1210 $10^6$ i.p. (A); in histocompatible $CD2F_1$ or incompatible $BD2F_1$ mice injected with LSTRA $10^6$ i.p. (B); in histocompatible BALB/c or incompatible B10.D2 mice injected with LSTRA $10^6$ i.p. (C).*

doses of the drug (180 and 300 mg/kg) died earlier than untreated controls of the same strain (Fig. 3C).

Experiments were performed to test the antineoplastic efficiency of vincristine in the three host-tumor systems under investigation. The results (Fig. 4) show that a single dose of VCR exerted limited antitumor effects in $CD2F_1$ mice carrying the compatible L1210 leukemia (Fig. 4A). The optimal dose of the antitumor agent (2.8 mg/kg) provoked an increase in MST of 40% over controls. In allogeneic BALB/c hosts VCR was highly effective over a wide dose range (i.e. from 0.216 to 1.65 mg/kg) as evidenced by the number of long-term survivors reported in Fig. 4A. Similar results were obtained when VCR was used in the LSTRA lymphoma model (Figs. 4B and 4C). VCR was more effective in allogeneic $BD2F_1$ or B10.D2 recipient mice than in compatible $CD2F_1$ or BALB/c hosts. However, the dose range leading to high percentages of long-term survivors among incompatible mice was more restricted in LSTRA-bearing $BD2F_1$ hosts than in L1210-bearing BALB/c mice (Figs. 4A and 4B). No exhaustive data is presently available in LSTRA-bearing B10.D2 hosts, since doses of VCR below 0.129 mg/kg i.p. were not tested (Fig. 4C). The majority of leukemic B10.D2 mice treated with this dose of VCR survived more than 60 days after challenge. Therefore it is reasonable to assume that lower doses of VCR might still be able to induce high percentages of long-term survivors.

Further studies were conducted in order to confirm that an immunological mechanism of host-anti-graft response underlies the increased efficiency of VCR in allogeneic tumor-host

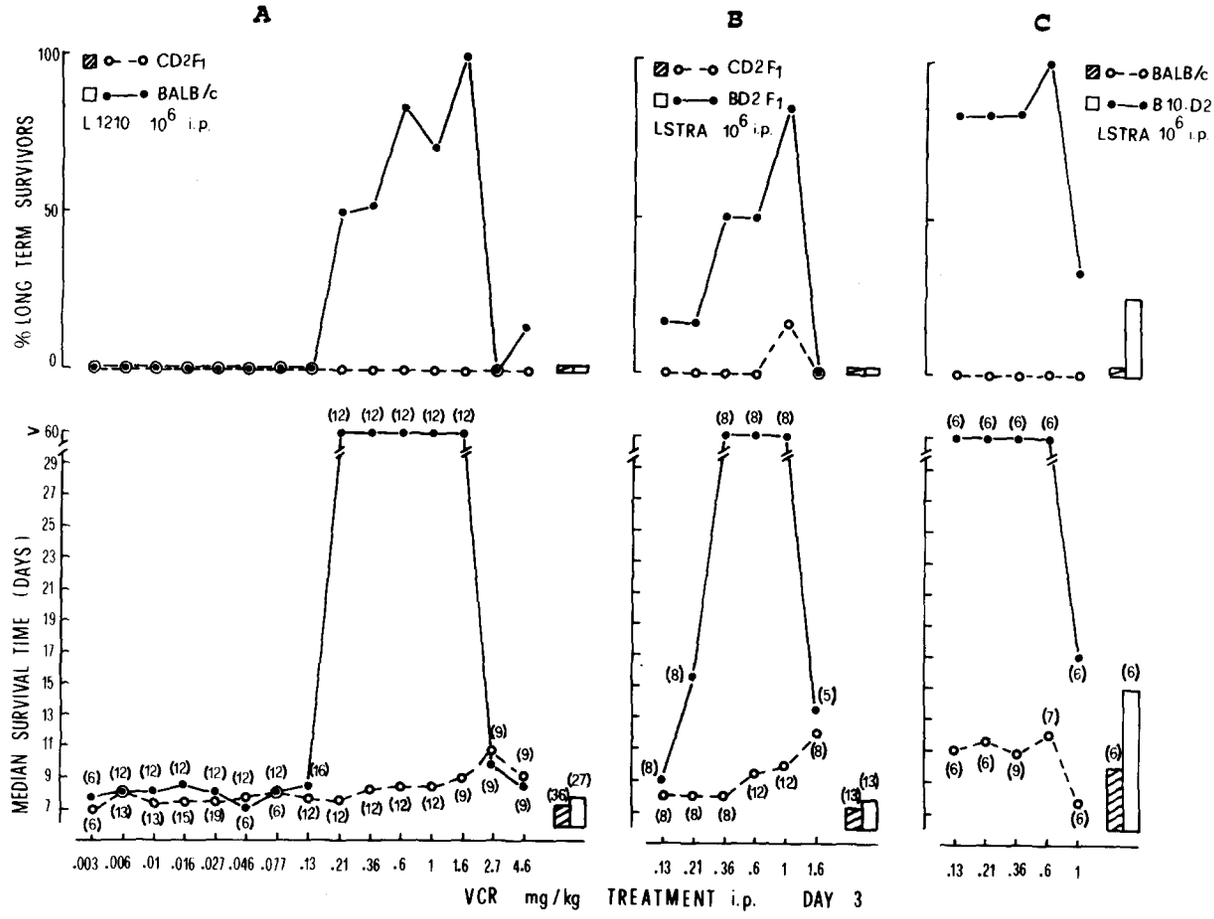


*Fig. 4. Antileukemic effect of graded doses of VCR, in histocompatible $CD2F_1$ or incompatible BALB/c mice injected with L1210 $10^6$ i.p. (A); in histocompatible $CD2F_1$ or incompatible $BD2F_1$ mice injected with LSTRA $10^6$ i.p. (B); in histocompatible BALB/c or incompatible B10.D2 mice injected with LSTRA $10^6$ i.p. (C).*

systems. Leukemia LSTRA ($10^6$ cells i.p.) was inoculated into compatible $CD2F_1$ or incompatible $BD2F_1$ recipients, either intact or immunodepressed by whole-body irradiation (400 rad) given 4–5 hr before tumor challenge. Three days later the mice were treated with a single i.p. injection of VCR (0.6 mg/kg). The results illustrated in Table 4. show that the anti-tumor effects of VCR were greater in non-irradiated allogeneic $BD2F_1$ mice than in compatible $CD2F_1$ recipients. However, the efficiency of VCR was reduced in pre-irradiated, immunodepressed $BD2F_1$, in which the anti-LSTRA allograft responses were weakened by the irradiation given before tumor challenge.

The last series of studies were conducted with HMM in the three experimental models under investigation. The results (Fig. 5) show that no antitumor effect was afforded by HMM given at doses ranging from 51.8 to 400 mg/kg i.p., either in compatible or MMHL-incompatible recipients, bearing both L1210 or LSTRA lymphoma.

## DISCUSSION

The studies described in the present report were designed to analyze the influence of anti-lymphoma immune responses on the efficacy of antineoplastic agents. The experimental model included host–tumor systems entirely histocompatible or incompatible for minor histocompatibility loci. In the latter situation, direct evidence was presented for immune reaction being mounted against lymphoma cells by presensitization experiments in which animals could be saved by administration of a tumor cell vaccine (Table 2). In general, host's responses against tumor-associated transplantation antigens (TATA) are most relevant to the clinical situation. Therefore, experimental immunochemotherapy studies

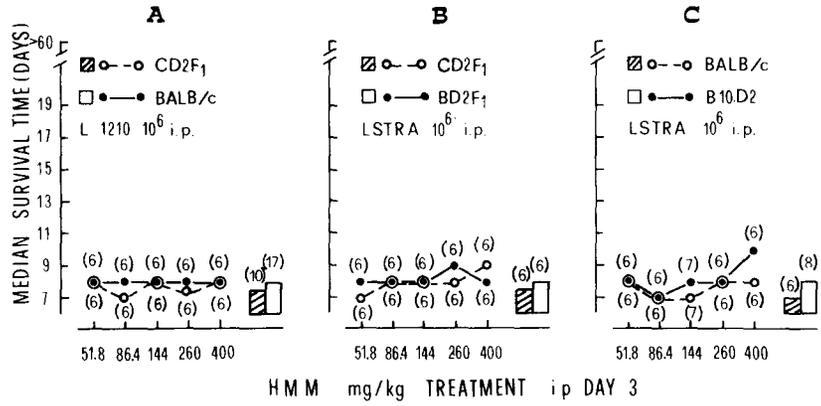


*Fig. 5. Antileukemic effect of graded doses of HMM, in histocompatible $CD2F_1$ or incompatible BALB/c mice injected with L1210 $10^6$ i.p. (A); in histocompatible $CD2F_1$ or incompatible $BD2F_1$ mice injected with LSTRA $10^6$ i.p. (B); in histocompatible BALB/c or incompatible B10.D2 mice injected with LSTRA $10^6$ i.p. (C). No long term survivors were found.*

*Table 4. Effect of immunodepression on vicristine activity against LSTRA leukemia**

| Host | Pretreatment† | Treatment‡ | MST | D/T |
|---|---|---|---|---|
| $CD2F_1$ | — | − | 7.5 | 10/10 |
| $CD2F_1$ | — | + | 9 | 8/8 |
| $CD2F_1$ | 400r | − | 7 | 8/8 |
| $CD2F_1$ | 400r | + | 8.5 | 8/8 |
| $BD2F_1$ | — | − | 8 | 7/7 |
| $BD2F_1$ | — | + | — | 4/8 |
| $BD2F_1$ | 400r | − | 8 | 8/8 |
| $BD2F_1$ | 400r | + | 8 | 8/8 |

*$10^6$ leukemia cells were injected i.p.
†Mice were irradiated 4–5 hr before tumor graft.
‡0.6 mg/kg of vincristine on day 3 after challenge.

have been concentrated on animal models in which anti-tumor immune responses were directed against TATA. Nevertheless, in the present investigation, combined effects of chemotherapy and anticancer immunity have been studied using tumor allograft systems in mice. Although host's responses against TATA may be quantitatively and qualitatively different from those directed against alloantigens, the allograft model was adopted for the following reasons: (a) there is general agreement that reactivity to alloantigens is related to reactivity against tumor-associated antigens. Although the cell-mediated immune response to neoplasia is quite complex, with evidence for involvement of T-cells, non-T-lymphocytes and macrophages [1, 9], it is widely accepted that, in terms of importance to *in vivo* resistance against tumor growth, T-cells appear to play a crucial role [12]. It has been shown in the FBL-3 mouse leukemia system that T-cells were needed for effective adoptive transfer [13]. Similarly, Glaser found that T-cells, and not complement receptor-bearing cells or macrophages, were essential for adoptive transfer of resistance against the rat leukemia (C58NT)D [14]. In three different tumor systems, rapid development of T-cell-dependent cytotoxicity after secondary tumor challenge has been demonstrated [15–17]. These findings support the contention that information gained with allogeneic models may provide insight into reactivity against tumor-associated antigens inasmuch as T-cells

appear to be the major cells involved in cytotoxic reactions against alloantigens; (b) in the allograft model differential effects of antineoplastic agents in the presence or in the absence of antitumor immunity can be studied using histocompatible or histoincompatible host–tumor systems. When similar studies are carried out in the anti-TATA model, the effects of combined immunochemotherapy must be evaluated comparing the antitumor efficiency of anticancer drugs in intact or in immunodepressed syngeneic mice. In this case, the agent used for impairing host's responsiveness could interfere with subsequent chemotherapy, leading to possible misinterpretation of the results. Alternatively, the effects of drugs in the presence or absence of antitumor graft responses can be studied using TATA-positive or TATA-negative tumors inoculated into non-immunodepressed mice. However, the sensitivity of two different tumors to the same drug is rarely identical, resulting in non-comparable chemotherapeutic effects; (c) when the allograft model is used, it is possible to define the extent of host's anti-tumor responses, selecting appropriate host–tumor genetic combinations. Therefore, anti-neoplastic agents can be tested against a single tumor line grafted into different strains of mice, in which the antineoplastic immune reactions are genetically determined; (d) large-scale screening of immunochemotherapy can be conducted with L1210 leukemia, a tumor currently used in the screening program for new antineoplastic agents [18].

In the allograft models described in the present communication, two different tumors, L1210 and LSTRA lymphomas, were used. They elicited weak and comparable allograft responses when inoculated into BALB/c or $BD2F_1$ recipient mice respectively, (Table 1), despite the fact that in the syngeneic situation LSTRA, which is a virus-induced tumor, may be considerably more antigenic. In addition, comparative studies were conducted using two different levels of allograft responses against the same tumor, when $BD2F_1$ or B10.D2 mice bearing LSTRA lymphoma were tested. In this case, B10.D2 were stronger responders than $BD2F_1$ hosts against LSTRA alloantigens, as evidenced by survival times reported in Table 1 or Figs. 1–5. The data of Fig. 1 confirm previous observations [8] that treatment with BCNU is highly efficient in lymphomas inoculated into weakly incompatible hosts (i.e. L1210 into BALB/c or LSTRA into $BD2F_1$ mice). When the drug was used at 50 mg/kg, the median survival times of the mice varied greatly in different experiments, as a result of variable numbers of toxic deaths. The studies carried out with Cy show that limited additive or synergistic effects between weak allograft responses and chemotherapy could be found in the L1210-into-BALB/c or LSTRA-into-$BD2F_1$ systems, in spite of a marked antitumor activity of the drug, detectable in compatible mice (Fig. 2). On the other hand, marked increase of drug efficiency was detected in LSTRA-into-B10.D2 system, in which stronger allograft reaction occurred. In B10.D2 mice toxic deaths played a major role in decreasing the MSTs at 180 and 300 mg/kg (Fig. 2).

The studies performed with DTIC showed that marginal or no increase of the effectiveness of drug treatment was detected in allogeneic mice, either in the L1210 or LSTRA system (Table 3, Fig. 3). When LSTRA-into-B10.D2 model was used, DTIC treatment at the dose of 163 mg/kg increased the MST of 5 days over that of untreated B10.D2 controls (Fig. 3). By increasing the dose of the drug, reduction rather than increase of MST was found, although 204 and 256 mg/kg were non-toxic doses. In addition, compatible BALB/c mice had higher MST than B10.D2 hosts when treated with the optimal dose (320 mg/kg) of DTIC. This observation points out that the direct antineoplastic activity of the compound cannot be the exclusive factor conditioning the effectiveness of chemotherapy in allogeneic mice. A balance between drug effects on the host (i.e., immunodepressive and/or toxic) and cytotoxic activity on tumor cells should be considered [18]. Therefore the results obtained with DTIC are in agreement with previous studies showing that this compound inhibits markedly allograft reactions, tumoral responses and T-mediated cytotoxic immune responses [19–21].

As opposed to DTIC, vincristine provides an example of a drug minimally active in histocompatible mice, but strongly efficient in the presence of even limited allograft reactions (Fig. 4). In addition, synergistic effects between chemotherapy and immune responses were always demonstrated when high but non-toxic doses of VCR were used. The role played by host's immune response in the increased efficiency of VCR in allogeneic mice seems to be confirmed by the experiments performed with irradiated animals. Chemo-immune collaborative activity did not occur in histoincompatible recipients immunodepressed by whole-body irradiation before tumor challenge (Table 4). It is hard to understand

the mechanism underlying immunochemotherapy synergism following administration of VCR, barely or not active against lymphoma cells in absence of anti-graft immune responses (Fig. 4, Table 4). It seems reasonable to hypothesize that VCR might be active on the host rather than on cancer cells, increasing host-anti-graft immune responsiveness, as suggested by recent *in vitro* experiments [22]. Evidence, indeed, to the lack of marked immunodepressive effects of VCR was provided by experiments where the drug, as opposed to Cy (Table 2), could not prevent the induction of immunity by a tumor cell vaccine in the LSTRA-into-BD2F$_1$ system (data not shown).

Finally, an essentially inactive drug such as HMM did not evidence appreciable antitumor effects either in histocompatible or allogeneic mice (Fig. 5). This finding could be interpreted in two ways: (a) HMM is totally inactive against lymphoma, tested at this treatment schedule and therefore, no synergistic effects with allograft reactions are expected; (b) strong immunodepressant activity of HMM could abolish the allograft response, leading to a sort of self-inhibition of the capacity of acting in combination with antitumor responses [23].

In conclusion, the results obtained in the present studies can be explained considering that host's factors (e.g., sensitivity to drug-induced toxic effects, or modulation of graft resistance) and tumor's factors (e.g., sensitivity to drug treatment) play a critical role in determining the efficiency of antineoplastic agents in mice bearing incompatible tumors. It is conceivable that drugs capable of producing synergistic effects with antitumor graft responses would be of great interest for clinical application, since antineoplastic immune reactions have been evidenced in man [9]. Therefore, the experimental models illustrated in the present report could be of value for selecting antineoplastic agents among drugs with comparable antitumor activity. This selection would be carried out taking into account the degree of efficiency in the presence of host's antitumor immune response.

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