Augmentation of Chemotherapeutically Induced Remission of a Murine Leukemia by a Chemical Immunoadjuvant

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SUMMARY

The effect of combining chemotherapy and immunostimulation, induced by a chemical, against a murine lymphoid leukemia was investigated. When tetramisole or levamisole were used following one administration of 1,3-bis(2-chloroethyl)-1-nitrosourea, a significantly higher percentage of long-term survivors occurred in contrast to the groups of animals treated with drug or immunostimulant alone. The administration of chemical immunostimulator was effective at any time during 1,3-bis(2-chloroethyl)-1-nitrosourea-induced remission. Low doses of the chemicals were as effective as higher doses in evoking this synergistic response. The role of chemical immunostimulators in combination with antitumor drugs is discussed.

INTRODUCTION

Chemotherapy of murine leukemia model systems often lead to long-term remissions and, in a few cases, to a cure of the animal's disease. However, best protection is often achieved either by early treatment, when the disease is not disseminated, or by chronic drug treatment. To date, very few drugs, when administered at tolerated doses, cause long-lasting remission and/or cures. If a specific drug can induce a long-lasting remission by effectively reducing the tumor load of the animals, then an ancillary therapeutic approach can be used to determine its ability to maintain and/or extend the remission period.

BCG¹ reportedly protects against transplantable (5, 9, 17, 26) and spontaneous (23) tumors, as well as against virus-induced tumors (7, 22, 24). In contrast, it also reportedly accelerates the growth of rat mammary tumors (14).

However, the use of BCG, as an immunoadjuvant in animals with a reduced tumor mass through chemotherapy has resulted in more effective therapy (1). Combined treatment with an effective remission-inducing drug with BCG proved even more efficient in prolonging survival and percentage survival of a lymphocytic leukemia (12). Furthermore, it was demonstrated that BCG administered during the early phase of drug-induced remission was more effective, and that the protective effect afforded by BCG appeared to be independent of a wide range of doses and route of administration. Doses containing as few as 80 to 800 viable BCG organisms were as effective as much higher doses (11).

The use of chemicals known to stimulate cellular or humoral immune responses has not been investigated to any degree to determine their effect when used in concert with chemotherapy in the treatment of leukemia. TMS and its l isomer, LMS, reportedly have a stimulatory effect on antibacterial immunization (18) and on anti-body-forming spleen cells in mice immunized with sheep red blood cells (19). The graft-versus-host reaction was also shown to be enhanced (20).

The more effective control of murine leukemia by combined chemotherapy and BCG immunostimulation prompted us to determine whether known chemical immunostimulation would be as effective as BCG.

MATERIALS AND METHODS

Tumor. A Moloney lymphoid leukemia line (LSTRA), originally induced in BALB/c mice by the Moloney murine leukemia virus, has been maintained and passaged routinely in our laboratory as a transplantable tumor line for over 300 generations in BALB/c × DBA/2 F₁ (hereafter called CD2F₁) mice. The ascitic tumor is serially transplanted i.p. at weekly intervals.

Mice. Adult CD2F₁, males, 8 to 12 weeks old, were obtained from the Charles River Breeding Laboratories, Wilmington, Mass. The animals were housed in plastic cages and fed Purina laboratory chow with water ad libitum.

Drugs. BCNU was kindly supplied by the Drug Development Branch, Division of Cancer Control, National Cancer Institute, NIH, Bethesda, Md. The alkylating agent was dissolved in a steroid-suspending vehicle and administered s.c. in a constant volume of 0.01 ml/g of body weight.

TMS and LMS were kindly supplied by Dr. P. Janssen, Janssen Pharmaceutica, Beerse, Belgium. The chemicals were dissolved in sterile 0.85% NaCl solution and administered i.p. in a constant volume of 0.01 ml/g of body weight.

Grafting Technique. The grafting technique used in this study is essentially the same as that used by Howard et al. (6). Briefly, skin grafts from C57BL/6 male mice were

¹The abbreviations used are: BCG, Bacillus Calmette-Guérin; TMS, tetramisole [d(-)-2,3,5,6-tetrahydro-6-phenylimidazo(+2,1-b)thiazole]; LMS, levamisole (l isomer); MST, median survival time; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

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placed on the thoracic wall of female C57BL/6 mice and covered with Vaseline petroleum jelly-impregnated gauze and a plastic cast. Casts were removed 7 days after grafting and the grafts were inspected daily for signs of rejection. Grafts were considered to be rejected at the first signs of hemorrhage or induration.

RESULTS

We have, in previous studies, established that adult CD2F₁ mice inoculated with 1 x 10⁴ LSTRA cells die with disseminated disease within 12 to 18 days. Treatment with BCNU (30 mg/kg) at a time that the disease is systemic results in a 10 to 12-day remission period, followed by relapse and eventual death of 70 to 80% of the treated animals (11, 12). The remission period was shown to extend up to the 19th day after tumor inoculation (11th day after drug treatment).

We thus focused our attention on treatment with chemical immunoadjuvant during the remission period, when the tumor mass was at a minimal level. The results in Chart 1 show that BCNU treatment extended the lifespan of the diseased animals from an MST of 13 days (Group 1) to an MST of 34 days (Group 3). The 1st death in the treated group occurred on the 20th day, at the time we have demonstrated relapse occurs. BCNU treatment was effective in retarding the disease; as a result of treatment 25% of the animals survived for more than 140 days without any symptoms of the disease. Treatment with TMS alone (Group 2) did not appear to exert any antitumor effect. However, when TMS was injected during the BCNU-induced remission period (Groups 4 to 8), a marked protective effect was achieved. In every group that received the combined treatment, a 60% or greater survival rate resulted. All animals that received treatment on the 15th day survived (Group 5). A single treatment with TMS appeared to be sufficient to produce the effect, since animals that received 4 treatments (Group 8) did not exhibit any greater percentage of survival.

Since TMS is the racemic mixture of the chemical, we tested whether the L-isomer would be any more effective (Chart 2). Again, BCNU treatment extended the MST from 15 days (Group 1) to 34 days (Group 2), with 25% of the animals surviving for more than 100 days. Treatment with a single dose of LMS did not alter the MST, although 1 animal in each of 2 groups (Groups 3 and 5) were alive at Day 100. Combined treatment again proved effective in sparing the animals from recrudescence of the disease. A greater than 80% survival rate was achieved in every group receiving combined treatment, except Group 7, in which 58% survival occurred. In 4 of the 6 groups, a greater than 90% survival rate was attained. There did not appear to be marked differences in total percentage survival between the groups receiving the DL or L form. In Groups 6 to 8, which received the L form 30/36, and in Groups 9 to 11, which received the L form 32/36, survival was greater than 100 days. These results confirm the synergistic effects attained by combining a remission-inducing drug (BCNU) with an immunoadjuvant (LMS or TMS).

The next study was designed to test whether a dose-dependent response would occur when the immunoadjuvant was administered at different doses at a time when the animals were in BCNU-induced remission (Chart 3). BCNU treatment effectively extended survival time, and
The results indicate that, at the doses tested, there does not appear to be a dose-dependent response. At 2.5 mg/kg, TMS appeared to be as effective as the 5 and 15 mg/kg doses. LMS at 2.5 mg/kg was slightly better than any of the higher doses of LMS tested.

The excellent results we achieved in combining BCNU and the chemical immunoadjuvant prompted us to confirm that TMS was indeed acting as an immunoadjuvant. Using the skin graft technique in C57BL/6 mice, we tested the effect TMS treatment would have on altering the time of graft rejection. The results (Chart 4) show that grafted mice treated with TMS at the time of grafting rejected their grafts at a significantly earlier time than controls. A higher percentage of mice rejected their grafts earlier when treated with either 20 mg TMS per kg or with 2 doses of 10 mg/kg. These results are indicative of a stimulation of the cellular immune response.

**DISCUSSION**

Very few chemicals have been used as immunoadjuvants in attempts to stimulate cellular or humoral immune responses in tumorous animals. One would expect that the tumor-bearing host would be immunologically depressed and therefore incapable of mounting any significant immune response, particularly in a disease involving the lymphatic tissues. However, if one could reduce the tumor mass of the host without excessively impairing the immunological competence of the host, then stimulation of host immune factors is possible. The success achieved with BCG implies that nonspecific immunity, when evoked, can retard or contain tumor growth.

Chemicals have been reported to act as immunoadjuvants. Two polyanionic compounds, polyribosinic-polyribocytidylic acid and pyran copolymer, reportedly stimulate cellular and humoral immune responses (2, 10, 16, 25). These agents, however, have also been shown to induce interferon or exert some antitumor activity (3, 8, 13, 15). Treatment with exogenous interferon has been reported to exert antitumor activity (4). The D₄ and L forms of phenylimidothiazole do not appear to exert their effect through interferon induction or direct antitumor activity. The immunological stimulatory effects reported by Renoux and Renoux (18–20), for these chemicals support this observation, and the observation that TMS-treated animals rejected their grafts at an earlier time indicates that the protective effect observed in these studies is mediated through a cellular immunity. In addition, Renoux and Renoux (21) demonstrated that LMS inhibited growth of the Lewis lung tumor and attributed this response to a cell-mediated immune response.

Whether the phenylimidothiazoles are evoking their tumor-inhibitory effect through a direct antitumor effect or an immunostimulatory effect must be studied further in great detail. Several studies are in progress to delineate the possible mechanism responsible for its effect.

However, the results of the study show that when TMS or LMS is administered during the period of drug-induced remission, a significant number of animals survive for long periods of time. The surviving animals appeared healthy, gained weight, and were vigorous and active. No gross symptoms of leukemia were seen when they were sacrificed at 100 or 140 days. Treatment at any time during the remission period with either the D₄ or L form, or at doses ranging from 2.5 to 15 mg/kg, was effective.

Chemicals such as reported in this study add to the list of potential stimulatory agents, such as BCG, that can be used in concert with chemoimmunotherapy. The use of chemicals, rather than biological materials (BCG and/or derivatives), presents several obvious advantages: possible side effects can be determined and avoided, assay methods for rapid quantitative measurement of the chemical can be developed, the site of action can be elucidated, structural activity relationships can be determined, and synthesis of more active congeners can be prepared.

These chemicals are presently being studied in other tumor systems (solid and lymphatic) to determine their immunoadjuvant activity.

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