The Combined Effect of Drugs and Tumor-specific Antibodies in Protection against a Mouse Lymphoma

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SUMMARY

In a model system of a mouse lymphoma growing in its syngeneic host, a measurable prolongation of life can be achieved with subtoxic doses of nitrogen mustard drugs and also with rabbit anti-mouse lymphoma cell sera that have been absorbed with normal mouse spleen cells so as to be noncytotoxic when tested on normal mouse lymph node cells as target. When antibody was injected 1 hr after the drug, a greatly improved result could be obtained. This synergism is not confined to nitrogen mustards; cytosine arabinoside followed by antibody in amounts where either alone gave the modest life prolongation of 12 and 4 days, respectively, gave 100% protection from tumor growth under the test conditions, even when the tumor escape time (between challenge and treatment) was extended to 96 hr.

INTRODUCTION

While the effectiveness of passively administered tumor-specific antibodies falls short of clinical usefulness in controlling tumor growth, their corresponding antigens remain the most obvious feature regularly found to distinguish between a tumor and its host, and the antibody is a strong candidate for an arrow to convey a poison to a tumor target. Poisons for such arrows that have been mooted, and even studied to some extent, are radiation emitters (7) and drugs.

The attachment of drugs to antibodies in such a way as to retain both antibody specificity and drug toxicity has been achieved in in vitro models (10), but the complexes do not act as “poisoned arrows” in vivo (5). Beneficial effects claimed so far in vivo are perhaps attributable to the separate actions of drug and antibody. This effect is synergistic and was shown for several nitrogen mustards using 2 different mouse lymphomas grown in their syngeneic hosts (3, 4).

This paper shows that the synergistic effect is not confined to nitrogen mustards and that the most effective drug when used alone is also the best one for cooperation with antibody.

The relevance of these results to clinical affairs resides in the promise of improving on passively delivered immunity for cancer patients who are, for reasons not properly understood, immunosuppressed (9, 12). This immunosuppressed state weighs against prospects for stimulating the active immunity, the failure of which might itself have allowed the tumor to grow. Only modest, although distinctly positive, results have nevertheless been obtained by nonspecific stimulation of immunity (Calmette-Guérin bacilli and corynebacteria) (11) and even by transfer of lymphoid cells from appropriately preimmunized animals (6).

MATERIALS AND METHODS

Animals. All mice were bred in our own colony and rabbits for immunization were bought from accredited dealers. The carcinogen-induced lymphoma EL4 is specific for C57BL/6 mice (8) and was maintained by i.p. passage in that strain.

Antisera. Groups of rabbits were immunized by 3 i.v. injections of 10⁶ EL4 cells at 10-day intervals and bled 10 days later. This particular schedule had been shown to provide an adequate titer of tumor-specific antibody (4). Sera were heated at 56° for 30 min and generally had a complement-mediated cytotoxic titer for C57BL/6 normal lymph node cells of about 1:1500. Sera were absorbed with normal mouse spleens until no cytotoxicity remained for normal cells (about 3000 to 5000 spleens/100 ml), but a small cytotoxic titer for EL4 cells was consistently retained (2). This is, of course, a very extravagant procedure with the sera available at this time.

Drugs. Chlorambucil and melphalan (Alkeran) were obtained from Burroughs Wellcome and Co., London, England, and cytosine arabinoside (1-D-arabinofuranosylcytosine) was from Sigma Chemical Co. Ltd., London, England. In the experiments described below these drugs were used at levels of 200 μg, 20 μg, and 5 mg, respectively. These doses are comparable in being about equally subtoxic, i.e., just less than the level that gives weight loss in the 4-dose treatment schedule used (3).

PT’s. Groups of C57BL/6 mice were challenged with 5 X 10⁴ EL4 cells i.p., unless stated otherwise; this is about 10⁴ lethal doses (4). “Treatment” consisted of 4 injections at 24-hr intervals, starting either 24, 48, or 96 hr after challenge, and involved i.p. administration of antiserum or drug, or both. Groups generally consisted of 10 or more mice, except where antiserum was given, when groups were limited to 5 animals because of the extravagance (in terms of spleens required for absorption) of tumor-specific serum. Results are plotted as percentage of survival. When both drug and antiserum were given, the injections were 1 hr apart but the order was drug-antibody, not the reverse, which does not work as well (3).

RESULTS

The Drug-antibody Effect with Melphalan. In this test (PT¹ 26) control mice died between the 13th and 18th days after

¹ The abbreviation used is: PT, protection test.
Chart 1. Protection of C57BL/6 mice against syngeneic EL4 lymphoma (10,000 lethal dose challenge). o—o, controls, 0.9% NaCl solution injections only; ∆—∆, normal rabbit serum only, with a time lapse of 48 hr between challenge and treatment. Treatment with absorbed rabbit anti-EL4 specific serum (0.5-ml, doses at 1:2 dilution): time lapse, 48 hr (○) and 96 hr (●); with melphalan (20-μg doses), time lapse, 48 hr (●). Treatment with melphalan (20-μg doses) followed 1 hr later by antiserum (0.5 ml doses at 1:2 dilution): time lapse 24 hr (●); 48 hr (○---○); and 96 hr (●---●).

Chart 2. Protection of C57BL/6 mice against syngeneic EL4 lymphoma. Conditions similar to those seen in Chart 1, but time lapse is 96 hr throughout. o, control mice, given injections of normal rabbit serum (0.5 ml); ∆, treatment with absorbed EL4 tumor specific xenoantiserum (0.5-ml doses); o, treatment with cytosine arabinoside (5-μg doses). Treatment with drug followed 1 hr later by antiserum (0.5-ml doses at 1:2 dilution), using chlorambucil at 200-μg doses (●); melphalan at 20-μg doses (●); cytosine arabinoside at 5-μg doses (●).
challenge and normal rabbit serum-treated mice gained no advantage over animals given injections of 0.9% NaCl solution (Chart 1, Curves A and B). Tumor-specific antiserum (batch R129/I31) in 0.5-ml doses at 1:2 dilution extended the life-span of mice by about 1 week when the time lapse between challenge and treatment was 48 hr (Chart 1, Curve C). When this “escape time” was extended to 96 hr the same amount of tumor-specific antiserum was no longer able to influence tumor growth (Chart 1, Curve D). Melphalan (phenylalanine-mustard) alone in 20-μg doses was also able to extend life-span by about 1 week with a time lapse of 48 hr (Chart 1, Curve E). With this 48-hr time lapse, drug followed each time (4 times at 24-hr intervals) by antibody 1 hr later gave a greatly enhanced effect (Chart 1, Curve G). Deaths began at about 40 days, but 60% of the mice survived indefinitely. The same treatment, but with a time lapse of only 24 hr, resulted in complete protection (Chart 1, Curve F), and even a 96-hr gap (Chart 1, Curve H) was impressive when compared with Chart 1, Curve D (same gap) and with drug action (not shown in the chart), which had about the same effectiveness as Curve D.

Drug-Antibody Mediated by Different Drugs. In the test (PT 32) illustrated in Chart 2, control mice treated with 0.5-ml doses of normal rabbit serum only, and with a time lapse of 96 hr between challenge and treatment, died between the 14th and 17th days (Chart 2, Curve A). Under similar conditions absorbed tumor-specific rabbit antiserum (R140/145) gave a small but significant prolongation of life of about 4 days (Chart 2, Curve B). Cytosine arabinoside alone at 5-mg doses gave protection to the extent of about 12 days, all mice having died by the 30th day (Chart 2, Curve C). Chlorambucil and melphalan were also tested at different dose levels alone, and 200-μg and 20-μg doses of these drugs, respectively, failed to show an effect at this extended time lapse when 96 hr were allowed for the tumor to become established. These data are omitted from the chart to preserve clarity but approximate to Curve A. This 200-μg dose level of chlorambucil followed 1 hr later by the same dose level of antisera as shown in Chart 2, Curve B, gave the synergistic effect seen in line D. In a similar situation, melphalan (20-μg doses) followed by antisera gave better results (Chart 2, Curve E), and cytosine arabinoside (5-mg doses) followed by antibody provided complete protection even at this 96-hr time lapse. The different levels of drug doses were approximately equally subtoxic, i.e., the chlorambucil and melphalan levels could not be increased with safety under the conditions used.

Whereas cytosine arabinoside is the drug to be favored in PT 32, it was no better than chlorambucil in mediating a drug-antibody effect in a somewhat similar test (PT 36) where a different lymphoma was used (SB1, in BALB/c mice). This test is not illustrated.

Challenge s.c. Although the dangers of emulating a test tube experiment by giving both challenge doses and treatment doses i.p. are minimized by the extended time lapses in the experiments described above, studies have also been made of s.c. challenge followed by i.p. treatment. Unexpectedly, it transpired that, using the same challenge doses, the i.p. route was the more difficult to protect against. With a 5 X 10^4 EL4 cell challenge dose given s.c. and allowing 10 days for tumor growth, which gave a solid tumor about 1 cm in diameter, treatment was started according to the 4-dose schedule previously described. In this case (PT 34) a 4-day delay from death by tumor was recorded using chlorambucil (200 μg) followed by antisera. Neither drug nor antiserum had any measurable effect alone.

DISCUSSION

In previous papers on this subject (3—5) we have shown that the effect of nitrogen mustard on neoplastic cells in an in vivo model can be greatly magnified if the injection of drug is followed by tumor-specific antisera or its immunoglobulin fraction. The antibody that was effective in the model used was originally from alloimmunization and absorbed in vivo (1, 5, 8), but the more clinically relevant xenoinmunization and in vitro absorption is now being used. From the results shown in Chart 1 we can see some limitations on melphalan and antiserum, in the sense that we cannot raise the drug level or easily raise the antiserum level. We could still extend the treatment schedule. However, the drug-antibody effect is easily seen in Chart 1, where the effect of the 2 components (Curve G) is not the sum of each given alone (Curves C and E) but is clearly synergistic.

The results in Chart 2 show that the drug-antibody effect is not confined to nitrogen mustards. Indeed, the most effective drug when we use comparable subtoxic doses is cytosine arabinoside, and this collaborates with antibody more effectively than any other drug tested so far in this system. The results in Chart 2, Curve F are infinitely better than those in Curves B and C. The choice of the best drug should be approached with caution, however, because it varies not only with different types of tumors but also among different mouse lymphomas.

That the antibody used is really tumor specific is confirmed by the thoroughness of our absorption checks (4). The location of this immunogen on tumor cell plasma membranes has previously been demonstrated (1), and this substance can be separated by gel filtration and ion-exchange chromatography from H-2 histocompatibility antigens and other alloantigens of EL4 cells for which there are test systems available (2). In addition, we have found a correlation between the titer of the antibody that locates this antigen on fractionation columns and the protective effect in in vivo tests (4).

In our earliest experiments, antibody was injected within 2 hr of challenge if any permanent survivors were to be expected (1). Using the drug-antibody effect, we are now able to allow 10,000 lethal doses of syngeneic tumor cells 96 hr to “settle in” and still obtain 100% survival. It is also possible to influence, although modestly, 10-day-old s.c. growths. The mechanism of this synergistic effect has not yet been clarified, but we suspect that antibody may be preventing recovery from drug damage. The system does provide specificity of action in spite of the systemic dispersal of drug. A drugged antibody might be more effective, but the linkages tested so far have failed to give specific effects in vivo (5) although they are effective in vitro (10).

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