Differential Lethal Effect of Cytotoxic Agents on Proliferating and Nonproliferating Lymphoid Cells

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SUMMARY

Dose-survival curves were constructed for the effect of eight cytotoxic agents on both nonproliferating and proliferating bone marrow-derived lymphocytes (B cells). The system used was the primary response of AKR mice to sheep red blood cells. Cytotoxic agents were given either 24 hr before or 24 hr after the antigen stimulation. These times were chosen to represent a time when the majority of B cells that would respond to sheep red blood cells were in a resting and a proliferating state, respectively. B cells were assayed for their ability to yield hemolytic plaque in vitro on Day 4. The degree of differential sensitivity and the shape of dose-survival curves for B cells in different proliferative states to these agents were, in general, compatible with the known cellular effect of these agents in other in vivo cell systems. The smallest difference in sensitivity was noted with γ-radiation and nitrogen mustard. Cytosine arabinoside, amethopterin, vincristine, and 5-fluorouracil had little or no effect on the survival of nonproliferating B cells whereas proliferating B cells were sensitive to these drugs. While nonproliferating B cells were sensitive to both cyclophosphamide and actinomycin D, proliferating B cells were far more susceptible to the cytocidal action of these two agents.

INTRODUCTION

Almost all the agents used in the treatment of neoplastic diseases and for the suppression of undesired immune responses are classified as "cytotoxic" drugs, that is, they are toxic not only to malignant cells but also to normal cells. The reason why these agents can be successfully used to treat cancer or inhibit the immune response without causing irreversible damage to other normal tissues is poorly understood. One explanation put forth by Bruce et al. (7) is that differences in sensitivity to cytotoxic agents are a consequence of the proliferative state of the cell populations in question. Rapidly dividing murine lymphoma cells are often far more susceptible to the cytocidal action of these agents than are resting hemopoietic stem cells (6, 7). The latter, however, can become as sensitive as lymphoma cells when they are induced to proliferate (5). Similar findings have been observed for mammalian cells grown in culture (2, 14, 18) and for bacteria (17) that exponentially growing cells were much more sensitive to these drugs than the same cells in a resting state.

Although much information is available concerning the effects of immuno-suppressive agents on various immune responses [see reviews by Gabrielsen and Good (10) and Makinodan et al. (15)], in only a few studies have the authors tried to explain and to correlate these effects in the context of the proliferative state of lymphoid cells (1, 19).

The primary response of AKR mice to SRBC2 has been studied extensively. B cells are the precursors of antibody-synthesizing cells and these cells are normally in a non-proliferative, G0 state (19). Within 12 hr of antigen stimulation, those B cells that are committed to make anti-SRBC antibody begin to proliferate and the majority of such cells are in a proliferative state by 18 hr (16). The number of IgM PFC increases above the background level by 24 hr and thereafter increases exponentially until 96 hr after antigen injection at which time the number of PFC begins to decline (16). The reason for this decline appears to be a consequence of the feedback inhibition by anti-SRBC IgG antibodies (21).

In this study, the primary immune response of AKR mice to SRBC was used as a model system to study the effect of cytotoxic agents on "resting" and "proliferating" B cells. The results indicate that the response of these lymphoid cells to cytotoxic agents can be predicted from the known cellular effects of these agents.

MATERIALS AND METHODS

Mice. Male AKR mice, 8 to 10 weeks old and weighing 20 to 24 g, from National Laboratory Animal Co., Creve Coeur, Mo., were used. Only male mice were used since from our own experience they generally gave a higher number of PFC than did female mice.

Immunization and Timing of Treatment and Assay. Mice in groups of 3 received 5 x 10⁷ washed SRBC per mouse as an i.p. injection on Day 0. Cytotoxic agents were given...
either 24 hr before (−24 hr) or 24 hr after (+24 hr) antigen injection unless otherwise stated. These times were selected to represent a time at which the majority of the B cells that would respond to SRBC stimulation were either in a resting state (−24 hr) or in a proliferating state (+24 hr). The number of direct PFC in the spleens was determined 4 days after the SRBC injection.

Hemolytic Plaque Assay. Spleens from the same treatment group were pooled and a monodisperse cell suspension was prepared by mincing spleens with scissors and passing the cells through a 120 mesh stainless steel screen with Medium α-minimal essential medium (20) that was buffered with morpholinopropanesulfonic acid and was adjusted to pH 7.2. A modification of the direct hemolytic plaque assay described by Jerne et al. (11) was used to enumerate the number of IgM PFC. The bottom layer in a 35-mm Falcon plastic Petri dish consisted of 1.0 ml of 0.5% agarose solution, 1 × 10⁶ SRBC in 0.1 ml and 0.1 ml of the spleen cell suspension appropriately diluted to give about 50 plaques per plate. Soon after the agarose had solidified, 0.3 ml of 1:5 diluted guinea pig complement (Grand Island Biological Co., Grand Island, N. Y.) was added to each plate. The plates were then incubated at 37° for 2 hr. Four to 6 plates were used for each cell suspension. The number of PFC was counted under a dissecting microscope and the results were expressed in terms of the immunized but untreated control as the fraction of PFC surviving per spleen. With this method, spleens from the control group contained 50,000 to 100,000 PFC per spleen on Day 4 and spleens from immunized AKR mice had a background count of less than 20 PFC per spleen.

Radiation of Mice. Mice were radiated (whole body) with a 137Cs unit (9) having a dose rate of 112 rads/min.

Drugs. Nitrogen mustard and actinomycin D were obtained from Merck, Sharp and Dohme, West Point, Pa.; 5-FU was from Roche Laboratories, Nutley, N. J.; CY was from Mead Johnson Laboratories, Evansville, Ind.; MTX was from Lederle Laboratories, Pearl River, N. Y.; ara-C was from Upjohn Co., Kalamazoo, Mich.; and VCR was from Eli Lilly and Co., Indianapolis, Ind. All drugs were diluted with pyrogen-free 0.9% NaCl solution and injected i.p. in a solution of 0.2 ml.

RESULTS

The timing of both treatment and PFC assay was important in examining the effect of these agents at the cellular level. The time interval of 24 hr was chosen for the −24-hr treatment to allow a sufficient time for the drug to be cleared from the plasma before the antigen was administered. For studying the effect of cytotoxic agents on proliferating B cell population, +24 hr was chosen since the majority of B cells that could proliferate and differentiate into anti-SRBC IgM PFC were dividing at the time (16).

Dose-survival curves for B cells to 8 cytotoxic agents were obtained and shown in Chart 1. The dashed line indicates the effect on resting B cells at −24 hr and the solid line illustrates the effect on proliferating B cells at +24 hr. All survival curves with the exception of γ-radiation were fitted by eye to the data from at least 3 separate experiments.

For studying the effect of γ-radiation on B cells, a single whole-body dose of up to 400 rads was given. The dose-survival curves indicate that the sensitivity of B cells to γ-radiation was not significantly affected by the proliferative state of these cells. The regression curves fitted to the data give D0 value of 72 and 80 rads for cells radiated at −24 hr and at +24 hr, respectively. These D0 values were comparable to those obtained for other lymphoid cells (8, 13). The effect of γ-radiation on B cells radiated at 3, 5, or 7 days prior to SRBC injection was also investigated. Dose-survival curves obtained from these experiments (not shown) were almost identical with those obtained at −24 hr and at +24 hr. These findings are in good agreement with the data reported previously (12).

B cells at −24 hr and at +24 hr were very sensitive to nitrogen mustard with cells at +24 hr slightly more sensitive to the drug especially at higher doses.

Very little or no killing of B cells was observed when ara-C, MTX, VCR, or 5-FU were given at −24 hr. In contrast, when graded doses of these drugs were given at +24 hr, there were significant reductions in the survivals. The shape of dose-survival curves for these drugs at +24 hr, however, differed considerably from each other. The survival to MTX decreased exponentially to 1% at a dose of 0.5 mg/mouse after which it decreased less rapidly. The dose-response curve for 5-FU given at +24 hr resembled that for MTX. With ara-C, 1 mg/mouse, given at +24 hr, the survival decreased to 25% of the control and no further reduction in survival was noted with higher doses. The shape of the dose-survival curve for VCR at +24 hr was quite different from those for MTX, 5-FU, and ara-C as it has an initial shoulder region followed by an exponential decrease in survival.

Both cells at −24 hr and at +24 hr were sensitive to actinomycin D and CY. Cells at +24 hr were definitely more susceptible to the lethal action of these 2 agents than cells at −24 hr, although the degree of differential sensitivity was far more pronounced with CY than with actinomycin D.

DISCUSSION

Our data indicate that the cellular response of B cells to cytotoxic agents can be significantly affected by the proliferative state of these cells. On the basis of the degree of differential sensitivity between proliferating and nonproliferating cells and whether they are active against the nonproliferating B cells, it may be possible to classify these agents into 3 groups. γ-Radiation is a representative of the 1st group of agents that kills both resting and proliferating B cells to the same extent. MTX, ara-C, VCR, and 5-FU belong to a 2nd group of drugs that have no or very little effect on the proliferative capacity of resting cells but are active against proliferating B cells. The 3rd group, consist-
Chart 1. Dose-survival curves for B cells to 8 cytotoxic agents given either 24 hr before (---) or 24 hr after (----) antigen injection. Spleens from immunized and treated mice were removed and assayed on Day 4 for their content of direct hemolytic PFC. The surviving fraction was obtained by normalizing the value to immunized but untreated controls. Open symbols, the values obtained from mice treated at -24 hr; closed symbols, values for those treated at +24 hr. Confidence intervals shown are 1 S.E. of the mean.
Chart 1—continued.

Charting of actinomycin D and CY, kills both resting and proliferating B cells but cells in the proliferative state are far more sensitive to these agents. Not every drug can be neatly classified into these 3 groups. Nitrogen mustard, for example, may be classified either with \( \gamma \)-radiation as a member of the 1st group for its small differential activity on
B cells at lower doses or with actinomycin D in the 3rd group. This grouping of these agents, in general, agrees fairly well with Bruce's (3) classification of cancer chemotherapeutic agents as nonspecific, phase-specific, and cycle-specific agents according to their cellular effect on mouse hemopoietic stem cells and lymphoma cells. There is, however, a difference in the classification of 5-FU. It belongs to the 2nd group of agents for B cells whereas it is classified as a cycle-specific agent by Bruce (3). This difference could be due to the type of cells used or due to the method used to construct the dose-survival curve for 5-FU: a single injection of drug for this study against multiple injections of 5-FU over a fixed period of time for theirs.

There are several factors that may affect the interpretation of these results. The most important one is a significant effect of these agents on macrophage and thymus-derived lymphocyte that are implicated in processing the antigen and in conveying the information to B cells. These steps are prerequisite in stimulating the B cells to proliferate and differentiate into antibody-producing cells (15). However, if this factor did play an important role, one would expect a greater influence of this effect on the survival obtained at −24 hr than at +24 hr. Since none of the agents used kills more cells at −24 hr than at +24 hr, it appears that the effects of these agents on the functional capacity of these 2 types of cells just mentioned are either negligible or they recover in time to perform their functions.

The influence of other factors such as feedback inhibition that occurs after Day 5 (21) and shifting of the time for the number of PFC in reaching its peak to after Day 4 or 5 as a consequence of the prolongation of PFC production after the insult (12) has been avoided by using Day 4 as the time for PFC assay.

With proper selection of timing for drug administration and for assay, we have shown that it is possible to use the primary immune response to SRBC in mice as a model to study the cellular effect of immunosuppressive agents and cancer chemotherapeutic agents on normal lymphoid cells. The system also offers the additional advantage of being able to study the cellular effect of various agents on both proliferating and nonproliferating B cells under almost identical conditions. Recently, a “cellular approach” to cancer chemotherapy was proposed (4). Since cytoxic agents are used for both cancer chemotherapy and immunosuppression, this system may be used in assessing the toxic effect of cancer chemotherapy regimens on host-immune responses; and furthermore, it opens up the possibility of using the similar “cellular approach” in choosing the right drugs, appropriate dosages, proper combinations, and correct timings for inhibiting undesired immune responses.

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REFERENCES

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