Synergistic Effects of Combination Sequential Immunotherapies in a Murine Ovarian Cancer Model

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ABSTRACT

The antitumor effects of Corynebacterium parvum in a murine ovarian teratocarcinoma model depend upon a sequential activation of neutrophils and macrophages within the peritoneal cavity. We studied the sequential administration of biological response modifiers that independently activate each phase of the response. Tumor-challenged mice treated by i.p. injection of a pyridine-extracted fraction of cell-free Propionibacterium acnes (PA-PE, 1400 µg) demonstrated prolonged survival in less than 20% of the cases. An i.p. injection of a detoxified Salmonella endotoxin (DSE) preparation (150 µg) had no effect on tumor outgrowth. However, i.p. treatment with PA-PE (1400 µg), followed by 150 µg of DSE 1 day later, resulted in long-term survival (>100 days) in 40 to 60% of mice. This antitumor effect was only evident when PA-PE was administered first (before DSE) and optimal when DSE was administered 24 h after PA-PE. The synergistic antitumor effect could be duplicated when tumor-challenged mice were first treated i.p. with peritoneal polymorphonuclear leukocytes, elicited by injection of PA-PE, and then treated with DSE 18 h later. These data indicate that appropriately timed injection of biological response modifiers with complementary effects can result in a synergistic prevention of tumor growth.

INTRODUCTION

A MOT3 which closely resembles disseminated ovarian cancer in humans has been used to study the antitumor effect of i.p. administration of a variety of BRMs (1-4). The i.p. injection of PA activates antitumor effector cells in the peritoneal cavity and results in prolonged survival of 75 to 95% of tumor-challenged mice. This antitumor response has been correlated with the early (within the first 24 h) generation of PMNs (5, 6), followed 2 to 3 days later by the appearance of tumor-cytostatic peritoneal mφs (6). In parallel clinical studies, i.p. administration of PA type I exerted significant antitumor effects in patients with ovarian cancer (7, 8). However, the effects were moderate, and the usefulness of the agent was limited by its toxic side effects.

To overcome these limitations, we embarked on studies to identify and isolate the component(s) of the bacteria responsible for its antitumor effects. The properties of a soluble bacterial cell fraction, obtained by exposure of PA type I cells to pyridine, have been examined. This isolation procedure caused the release from the cell surface of a preparation, identified herein as PA-PE, which is 70 to 80% carbohydrate in nature (2, 9). Initial studies suggest that this fraction may be less toxic than whole unfractionated PA organisms (9).

Our initial studies revealed that mice, inoculated with PA-PE after a 105 inoculum of MOT tumor cells, exhibited a powerful, albeit brief, antitumor response during the first 48 h, after which the tumor began to grow exponentially, killing 80 to 90% of the mice. Because the antitumor effects of PA-PE are manifested primarily during the first 48 h, we postulated that this agent was deficient in the capacity to generate the second cellular component, i.e., cytostatic mφ, involved in the tumor rejection process. Also, in another murine system, Chapes and Haskill (10) have demonstrated that PA-PE is ineffective in activating antitumor mφs. We hypothesized that an appropriately timed injection of a second BRM that activates mφs would greatly enhance the antitumor effects of PA-PE. As potential mφ activators, an endotoxin preparation that had been detoxified (DSE) (11-13) was utilized. The results of this study confirm our hypothesis that the administration of DSE following an injection of PA-PE causes a marked augmentation of the in vivo antitumor effects induced by the latter drug alone.

MATERIALS AND METHODS

Tumor. The MOT is a syngeneic tumor maintained by serial i.p. transplantation into C3HeB/FeJ mice every 2 wk. Tumor cell viability was assessed by trypan blue exclusion. An i.p. injection of as few as 103 MOT cells killed all recipient mice.

Mice. Female C3HeB/FeJ mice were obtained from Jackson Laboratories, Bar Harbor, ME, and fed a standard laboratory diet.

Experimental Method. Groups of 10 mice per dose were utilized. At the age of 8 to 10 wk, the mice were given injections of 105 tumor cells, treated i.p. with various agents 24 h later, and observed daily for survival. In experiments where agents are given sequentially, the second is administered i.p. 24 h after the first agent, equivalent to 48 h after i.p. tumor inoculation.

PA. A strain of PA and its biochemically derived fractions used in these experiments were obtained from Ribi Immunochem Research, Inc. (Hamilton, MT). PA-PE and PA-R fractions of both strains were prepared from freshly grown organisms as previously described (9, 14). The pyridine extract (PA-PE) is free of detectable cells or cell walls by electron microscopy and does not induce hepatomegaly or splenomegaly (9). PA-PE has antitumor activity (9) and augments natural killer cell activity (15).
Unlike PA-PE, the PA-R retains the ability to cause organomegaly (9).

DSE. The isolation of a nontoxic lipid A fraction of Salmonella typhimurium which retains tumor regression activity has been previously described (11, 12).

Peritoneal Lavage and Tumor Cell Counts. Methods of murine peritoneal lavage and tumor cell enumeration have been previously reported (5). Mice were sacrificed and lavaged with 10 ml of phosphate-buffered saline. Withdrawn peritoneal cells were washed and stained with Wright-Giemsa. Total tumor cells were determined by multiplying total peritoneal cell yield by the percentage of tumor cells.

Statistical Analysis. Survival curves are based on the product-limit estimated (Kaplan-Meier) (16), and equality in survivorship between groups is tested using the Breslow and Mantel-Cox tests (17).

RESULTS

PA-PE and Early Antitumor Effects That Are Not Sustained. Previous studies (2) have indicated that, on a per weight basis, PA-PE exerted a greatly reduced antitumor effect when compared to whole organisms in mice inoculated with 10⁵ tumor cells. Chart 1 demonstrates that this agent induces an early antitumor effect which is not sustained. After injection of 1400 U.Q of PA-PE, rapid tumor cytoreduction ensues. This effect is quantitatively comparable to that achieved with whole organisms (1–3). However, after a short lag period, tumor growth becomes reestablished, leading to the eventual death of 80% of the tumor-inoculated mice.

Combined Administration of PA-PE and DSE and Synergistic Antitumor Effects. As shown in Chart 2, none of the mice demonstrates prolonged survival after the i.p. inoculation of 700 µg of PA-PE. Similarly DSE given in a dose of 150 µg, 24 to 48 h after inoculation with 10⁵ tumor cells, has no discernible antitumor effect. In sharp contrast, when tumor-challenged mice are given 700 µg of PA-PE, followed by 150 µg of DSE 24 h later, a marked antitumor effect is seen (Chart 2), and survival is comparable to animals given injections of whole C. parvum in our laboratories (2). The augmentation of the antitumor response caused by the sequential administration of PA-PE and DSE is directly related to the dose of PA-PE administered (Chart 3).

If the sequence is reversed (DSE injected first), the augmentation of the antitumor effect, as well as the modest effect seen when PA-PE is given alone, is lost (Chart 4). In addition, the...
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Chart 4. Survival curves of mice given injections i.p. of 10^5 MOT, followed 24 h later with 1400 μg of PA-PE and thereafter with DSE (150 μg) 24 h later (C). 48 h later (H), and 72 h later (A). DSE (150 μg) is given 24 h after 10^5 MOT (A) and thereafter 24 h with 1400 μg of PA-PE. The survival curve with a 24-h difference between the two agents (PA-PE prior to DSE) is unique compared to control. 72-h intervals and the reverse order (DSE prior to PE-PE) (P < 0.01). The survival curve with a 48-h difference is unique compared to control and other curves (P < 0.05). Each survival curve represents ten inoculated mice.

optimal interval between injections of PA-PE and DSE is 24 h. If DSE is given 48 or 72 h after PA-PE administration, a progressive loss of the enhanced antitumor effect was observed.

Antitumor Effects between PMNs Elicited by PA-PE and a Single Injection of DSE. We hypothesized that the marked antitumor response that results from the sequential administration of PA-PE and DSE depended upon an interaction between the PMNs elicited by PA-PE and DSE. To gain support for this notion, we examined the capacity of PMNs obtained from mice inoculated with PA-PE (1400 μg) to control the growth of a lethal inoculum of MOT targets when adoptively transferred into naive mice at an effectortARGET ratio of 250:1. This effect was compared to the effect obtained when tumor cells are given alone and followed 18 h later by an injection of DSE. Chart 5 demonstrates that PA-PE-elicited PMNs are ineffective by themselves when injected with either a 10^6 or 10^4 tumor inoculum into naive mice. DSE injections by themselves have no effect against a 10^4 inoculum and minimal effects against a 10^3 tumor challenge. However, when DSE is injected 18 h after the inoculation of PA-PE-elicited PMNs, a marked antitumor effect was detected.

DISCUSSION

The use of better purified and characterized BRMs with more selective effects on host antitumor responses represents a goal of the biological therapy of cancer. This is particularly true for BRMs of bacterial origin. The latter are usually associated with significant toxicity which limits their applicability to human cancers.

Early experience with PA-PE suggested it was a desirable antitumor agent (2). The drug could activate host effector cells and induce antitumor effects in vivo (5, 6, 15). Furthermore the PA-PE fraction had been rendered free of the bacterial components responsible for the causation of side effects such as hepatosplenomegaly, fat deposition in the liver, and the generation of suppressor m0s (9, 12).

The results of this present study, however, indicate the pitfalls associated with the use of bacterial fractions with more selective activities. Most responses to tumor are complex, requiring sequential interactions between various cells and their mediators. Thus BRMs that elicit only a portion of the necessary sequence may not produce tumor rejection. In the MOT model, the polyfunctional unfractionated PA-type organisms successfully achieve tumor rejection, because they initiate a cascade of events. The induction of an acute inflammatory response leads to the sequential activation of tumoricidal PMNs, which is followed by the activation of macrophages within the peritoneal cavity. Although PA-PE exerts more selective biological effects and its side effects are minimized, an effective antitumor action is absent.

To counter this limitation, we chose to use PA-PE in combination with other BRMs known to be effective as m0 activators. The utilization of sequential immunotherapy was based on the notion that, once tumor rejection was initiated, each step in the cascade could be effectively engaged by the appropriately timed administration of specific agent(s). Thus PA-PE was used to selectively initiate inflammation, activate tumoricidal PMNs, and induce m0 influx, while DSE was used to activate tumor cytostatic peritoneal m0s (16). When used in combination, these drugs achieved tumor rejection which was comparable to that seen with 1400 μg of PA and significantly greater than that achieved with either agent alone.

It is unclear why DSE is ineffective in this tumor model when injected alone. One possibility is that the first phase of tumor rejection, which involves generation of tumoricidal PMNs, may
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be deficient following injection of DSE. Indeed the degree of neutrophilic exudation induced by DSE and the level of tumoricidal activity within the elicited PMNs are less than those achieved with PA-PE or unfractionated PA (unpublished data). A second possibility is that m0 activation achieved by injection of DSE is substantially increased when the drug is given soon after an initial injection of PA-PE. Studies are under way to better elucidate the cellular and biochemical mechanism(s) responsible for augmentation of antitumor effects when PA-PE and DSE are given sequentially to mice receiving a lethal tumor inoculum.

REFERENCES

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