Inhibitory Effect of Endotoxin on the Growth of Plasma Cell Tumor

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SUMMARY

Small amounts (0.1 ng to 5.0 μg) of Escherichia coli endotoxin protect normal female BALB/c mice against challenge with low doses of syngeneic mineral oil-induced 315 plasma cell tumor. Significant protection was most evident when mice were treated with endotoxin 11 days and 5 days before inoculation with 50 to 100 tumor cells i.p., and endotoxin treatment continued twice a week for the entire experiment. Tumors induced by 10,000 cells s.c. were similarly affected by this treatment. The antitumor action of endotoxin was obliterated when higher challenges of tumor cells or solid tumor pieces were used. Omission of endotoxin pretreatment resulted in a loss of the effect against i.p.-induced tumors but not against s.c.-induced tumor.

INTRODUCTION

Endotoxin can affect antibody synthesis (6-9, 14), activate macrophages (1, 2, 10), play a role in plasma cell tumor induction in oil injection-treated BALB/c mice (4, 12, 16), invoke a serum PCT* colony-stimulating factor (13), and cause regression of tumors (3, 9, 15, 18). In preliminary experiments (4), we found that chronic administration of ng quantities of endotoxin stimulated PCT formation in oil injection-treated mice. In this study, we have investigated the influence of low doses of endotoxin on the growth of transplanted PCT in normal mice. Under appropriate experimental conditions tumor growth can be prevented by minute amounts of endotoxin.

MATERIALS AND METHODS

Female BALB/c mice, 6 to 8 weeks old (Mammalian Genetics and Animal Production Section, Drug Research and Development, National Cancer Institute), were used throughout these experiments. The mice were maintained on Purina laboratory chow and water ad libitum. MOPC 315, a gift of Dr. Herman Eisen (Massachusetts Institute of Technology, Cambridge, Mass.), was used in all experiments. At least 2 tumors, 19 to 22 days after trocar transplantation, were minced in HBSS (Grand Island Biological Co., Grand Island, N. Y.). The resulting cell suspension was centrifuged at 137 × g for 5 min, washed once in fresh HBSS, and layered on 1.5 ml of a Ficoll-Hypaque gradient (5), density 1.088 g/ml in 13- × 100-mm test tubes. The tubes were centrifuged at 400 × g (at the interface of the gradient and cell suspension) for 20 min (International Equipment Co. clinical centrifuge) at room temperature. This procedure removed both RBC and nonviable tumor cells. The cell layer was collected from the interface, washed twice in fresh HBSS, and resuspended in HBSS. Cells were counted in a hemocytometer; cell viability (trypan blue exclusion) was 90 to 96%. Appropriate dilutions were made in HBSS from now on, and 0.5 ml of the cell suspension was used to inoculate tumor cells i.p. Tumors were induced s.c. either with 0.2 ml of the appropriate cell dilution or by trocar transplantation of approximately 1-mm tumor pieces.

E. coli endotoxin 055:B5 (Difco Laboratories, Inc., Detroit, Mich.) was prepared in sterile 0.15 M NaCl at appropriate concentrations and kept frozen until use. Acid-insoluble protein measured in 2- to 4-mg/ml solution was less than 1%. Control mice were sham-inoculated with a 25-gauge needle without fluid. Initially, 0.15 M NaCl was used in the pilot experiment. This was discontinued because of the possibility of injecting minute amounts of endotoxin. Four endotoxin treatments were compared. In Protocol 1, groups of mice were given i.p. injections of either 0.1 ng, 1.0 ng, 5.0 ng, or 5.0 μg endotoxin in 0.1 ml 6 days apart. Five days after the 2nd injection, the tumor was inoculated i.p. or s.c. Within 24 hr of tumor, the animals were given i.p. endotoxin injections twice a week; these injection patterns were continued for the duration of the experiment. Protocol 2 was identical to Protocol 1, except for the omission of the 2nd endotoxin injections prior to tumor inoculation. In Protocol 3, mice were inoculated with 1.0-mm pieces of MOPC 315 s.c. and injected s.c. within 24 hr with either 0.1 ng, 1.0 ng, 100.0 ng, or 10.0 μg endotoxin at the site of the tumor. The injections were given 3 times a week for the length of the experiment. Protocol 4 was identical to Protocol 3, except that tumors were allowed to become palpable before endotoxin treatment was started.

Autopsies were performed on Day 30 or when 75% of the sham-injection-treated animals had developed tumor. Tumor weight was obtained for each animal and calculated as present body weight of tumor. Ascites fluid, if any, was collected and measured. Significance of tumor inhibition by endotoxin was evaluated by the χ² test with Yates’ correction.

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4 The abbreviations used are: PCT, plasma cell tumor; MOPC, mineral oil-induced plasma cell tumor; HBSS, Hank’s balanced salt solution.
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RESULTS

Table 1 shows that pretreatment with endotoxin significantly inhibits tumor growth in animals challenged with either 50 to 100 tumor cells i.p. With 50 cells, 75% (6 of 8) of sham-injection-treated animals developed tumor by Day 30. In contrast, only about 11% (1 of 9 (p < 0.05)) of mice treated with 5.0 μg endotoxin developed tumor 30 days after challenge; ng amounts of endotoxin were not effective against this dose of tumor. However, ng amounts against 100 tumor cells, 1.0 ng and 5.0 ng endotoxin, reduced tumor incidence to 44.0% [8 of 18 (p < .005)] and to 61.0% [11 of 18 (p < 0.5)], respectively. μg amounts were inhibitory as well when compared to the sham- or 0.15 M NaCl injection-treated control group. There is no significant difference in the inhibition obtained with μg as compared to ng amounts of endotoxin. Prevention of tumor by endotoxin pretreatment was lost as the challenge dose of tumor cells was increased. All doses of endotoxin were ineffective against challenge with 5 × 10^5 to 10^6 tumor cells.

Animals in endotoxin-treated groups without cross-evidence of tumor showed no microscopic evidence of residual tumor. Tumors that did form in the experimental groups were examined and weighed. No statistical difference in the weight of tumor formed per group was found. Tumor nodules found in the peritoneal mesenteries were not necrotic nor were hemorrhagic areas clearly definable.

Endotoxin pretreatment was tested against s.c.-induced tumors to determine whether tumor cells had to be in direct contact with endotoxin as in the ascites system for inhibition to occur. To transplant MOPC 315 s.c. at least, 10,000 cells must be inoculated to produce 70% tumor by Day 30. Treatment with endotoxin (Protocol 1) again caused a significant inhibition of tumor growth in animals challenged with 10^3 cells (Table 2). Tumor developed in 14.3% [5 of 35 (p < 0.005)] of mice treated with 1.0 ng endotoxin. All doses of endotoxin tested were effective. Only 36% to 43% of mice treated with these amounts of endotoxin had tumor by Day 30. Examination of the tumors at autopsy did not disclose an increase in necrosis or hemorrhagic areas. The mean weight of tumor formed in the treated groups was not statistically different from control values.

Omission of endotoxin pretreatment resulted in a loss of the preventative effect against i.p.-injected tumors. All animals given endotoxin after transplantation of 100 or 10,000 cells i.p. develop tumor by Day 30. The mean weight of tumor formed by mice given 1.0 ng endotoxin and 100 tumor cells was reduced to 4% of the total body weight (p < 0.001) compared to 10.0% mean tumor weight obtained for control mice. However, elimination of endotoxin pretreatment did not completely obliterate the antitumor action of endotoxin against s.c.-induced tumors (p < 0.01; Table 3). Forty % (6 of 15) of mice given 10,000 tumor cells s.c. and 5.0 ng endotoxin developed tumor by Day 30 in contrast to 93% (14 of 15) of the sham-injection-treated animals. All other doses were ineffective in inhibiting tumor. Endotoxin in 5.0-μg amounts suppressed tumor in at least 47% of mice challenged with 100,000 cells (p < 0.01).

Injection of endotoxin directly at the site of the tumor did not prevent the outgrowth of transplanted tumor (Protocols 3 and 4). All animals developed tumor regardless of whether endotoxin was started within 24 hr of tumor challenge or when tumor became palpable. In the latter case, shrinkage of the tumor did not occur as determined by measurement of tumor diameter. In either case, no observable difference in the amount of necrosis between control and treated tumors was seen.

**DISCUSSION**

These experiments were designed to explore our preliminary observation that endotoxin stimulated PCT proliferation (4). We were surprised to find that small PCT inocula were not effective in normal mice, following a specific protocol of endotoxin treatment. Tumor growth was most
successfully prevented in both i.p. and s.c. tumor systems by pretreatment with endotoxin. Pretreatment with endotoxin is necessary to prevent tumor growth in other tumor systems (9, 18). No observations at the low doses reported here have been made.

ng as well as μg amounts of endotoxin were effective in inhibiting tumor. This range of effective dose indicated to us a strong dependence for the effect upon a variety of interrelated factors such as precise calibration of the inoculum, factors controlling growth of tumor at i.p. and s.c. sites, the influence of endogenous endotoxin as well as responsiveness of the immune system of the host to stimulation by exogenous antigen. We are particularly disturbed by the lack of a dose response to endotoxin. We believe that endogenous endotoxin and contamination of reagents with minute amounts of endotoxin play a role in concealing the dose response. This latter problem explains our preference for sham injection (insertion of a needle into the peritoneal cavity without fluid injection) as a control, although we show that 0.15 M NaCl injections are inactive. We are currently investigating methods for purifying reagents from ng quantities of endotoxin.

We have not demonstrated a mechanism of action for the endotoxin effect. Rejection of syngeneic PCT can be mediated by cytotoxic thymus-derived cells (18), or by antitumor antibody, known to be effective against small tumors (11). Endotoxin can facilitate both of these responses (7, 8) in addition to a nonspecific stimulation of immune cells (1, 2, 6).

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REFERENCES

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