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Influence of Steroidal and Nonsteroidal Sex Hormones on Host Resistance in Mice: Increased Susceptibility to *Listeria monocytogenes* After Exposure to Estrogenic Hormones

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Subchronic exposure to pharmacological levels of estrogenic compounds, including 17β-estradiol, diethylstilbestrol, and α-dienestrol, significantly increased the mortality of B6C3F$_1$ female mice after *Listeria* infection. Compounds with little estrogenic activity, including 5α-dihydrotestosterone, progesterone, zearalenol, and corticosterone, did not alter *Listeria*-related mortality. Estrogen-induced alterations in resistance were inhibited by both adult thymectomy and the estrogen antagonist tamoxifen. Estrogen exposure depressed the accumulation of monocytes and lymphocytes at infective foci. Significantly elevated numbers of bacteria were observed in infective foci of estrogen-treated mice later in the infection when bacteria were nearly eliminated from untreated animals. These results indicate that estrogen-induced suppression of *Listeria* immunity is partially mediated by the thymus, probably through receptor events which may ultimately suppress the activation of T cell-dependent defense mechanisms. This may be partially reflected by the inability of estrogen-exposed mice to eliminate *Listeria* cells or to accumulate mononuclear effector cells at infective foci at the same rate as controls.

Pharmacological (i.e., therapeutic) levels of estrogen alter resistance to a variety of infections in laboratory animals. For example, estrogen-treated mice are less susceptible than controls to bacteria such as *Pneumococcus* type I, *Pasteurella* spp., and *Salmonella* spp. (30). Mice exposed to diethylstilbestrol (DES) are more resistant to the formation of transplantable lung melanoma tumors (8, 29), and estrogens may also lessen the severity of certain plasmodial, babesial, and trypanosomal infections (2, 17, 26). Paradoxically, exposure to pharmacological levels of estrogen profoundly decreases host resistance to a variety of other infectious agents. Illustrative of this are the findings that DES exposure precipitates a dramatic increase in *Listeria* susceptibility, impairs the intestinal expulsion of adult *Trichinella* sp., and increases the susceptibility of mice to both viral and methylcholanthrene-induced tumors (4, 15). 17β-Estradiol induces more intense chlamydial (36) and staphylococcal (47) infections, whereas hexestrol administration increases the severity of experimental toxoplasmosis in mice (18).

The divergent effects of estrogens on resistance may, in some instances, be due to the quantity of chemical injected (8) or the route of infection (29). In addition, it is likely that altered host resistance correlates with immune function disturbances caused by estrogenic compounds. For example, estrogens are potent stimulants of the mononuclear phagocyte system (1, 22, 31, 49) but suppress natural killer cell activity (14, 41) and T cell-mediated immunity as well (12, 13, 23). Estrogens may induce these alterations by affecting the production of immunoregulatory thymic hormones (9, 24, 42). Increased resistance to certain organisms is often attributed to estrogen-related enhancement of macrophage activity (2, 17, 29, 30). Conversely, estrogen-induced defects in natural killer cell functions and T-cell responses may decrease resistance to other infectious agents (4, 15, 18).

The aim of the present study was to systematically examine the mechanisms associated with the increased susceptibility to *Listeria monocytogenes* infection which occurs after exposure to pharmacological levels of estrogen. The relationship between altered host resistance and the estrogenicity (i.e., uterotrophic activity) of sex hormones was determined by investigating the effect of a variety of steroidal and nonsteroidal compounds, with a range of estrogenic activities, on host resistance to *L. monocytogenes*. The role of the thymus and the operational estrogen receptor was studied by investigating the effects of adult thymectomy (TX) and the estrogen antagonist tamoxifen on *Listeria* resistance after estrogen exposure. In addition, studies were designed to determine the nature of the immune disturbances induced by estrogens which are responsible for the alterations in host resistance.

MATERIALS AND METHODS

Mice. Specific-pathogen-free B6C3F$_1$ (C57BL6 × C3H/He) female mice (6 to 8 weeks old) were obtained from National Cancer Institute production contracts (Charles River, Portage, Mich.). Animals weighed an average of ca. 20 g at the time of the studies, were maintained on a 12-h light-dark cycle at 68 to 73°F, and were provided with sterile food (Ziegler Bros., Inc., Gardner, Pa.) and water ad libitum. Thymectomies and ovariectomies were performed under pentobarbital anesthesia.

Dosing regimen. DES, 17β-estradiol, 5α-dihydrotestosterone (5α-DHT), α-dienestrol, and progesterone were obtained from Sigma Chemical Co. (St. Louis, Mo.). Corticosterone was obtained from Calbiochem-Behring (La Jolla, Calif.). Zearalenol was generously provided by M. Bachman

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of International Minerals and Chemical Corp. (Terre Haut, Ind.). β-Dienestrol was kindly donated by M. Metzler, University of Warzburg, Warzburg, Federal Republic of Germany. All chemicals except zearalenol and corticosterone were dissolved in corn oil with gentle heating. Zearalenol was dissolved in ethanol and mixed with corn oil after which alcohol was evaporated by blowing gaseous nitrogen over the mixture. Corticosterone was injected as a well-mixed suspension in corn oil. Chemical concentrations were prepared on a milligram per kilogram of body weight basis. Mice were treated with 2.8 μmol or less of chemical to minimize possible toxic or nonhormonal effects. Mice were injected subcutaneously in the dorsal cervical region with the desired concentration of chemical in 0.1 ml of corn oil for 5 consecutive days. Control mice received corn oil only (vehicle control). Studies were begun 3 to 5 days after the last treatment dose. In studies in which estrogen antagonists were examined, mice were dosed subcutaneously with 1.2 μmol of tamoxifen citrate (a gift of D. H. McCurdy, Stuart Pharmaceuticals, Wilmington, Del.) in saline daily for 3 days. On days 2 and 3 they also received 1.2 μmol of estradiol. Controls received saline and corn oil (vehicle control). In all cases, reported doses reflect the total amount of chemical injected.

***Listeria* susceptibility.** A frozen stock of *L. monocytogenes* (strain L242/73 type 4B) from a naturally infected mouse was used throughout this study. Bacteria were grown overnight at 37°C in brain heart infusion broth. The number of bacteria in the broth was quantitated turbidimetrically in a spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) at 540 nm and confirmed by plate counts. The desired concentration of bacteria was prepared by diluting the stock in cold Hanks balanced salt solution. Organisms were injected into mice intravenously (i.v.) in a volume of 0.2 ml 4 to 5 days after estrogen exposure. For survival studies mice were inoculated with ca. 5×10^4 viable *Listeria* cells (20% lethal dose) and monitored for 14 days or until death. Bacterial enumeration in spleens and livers was conducted after a nonlethal i.v. infection (10^4 organisms) as described previously (48). Briefly, a tissue grinder was used to homogenize organs in distilled water plus 0.05% Triton X-100 (New England Nuclear Corp., Boston, Mass.). Homogenates were serially diluted and plated on brain heart infusion agar plates. Triton X-100 was added to the diluent to lyse any intact

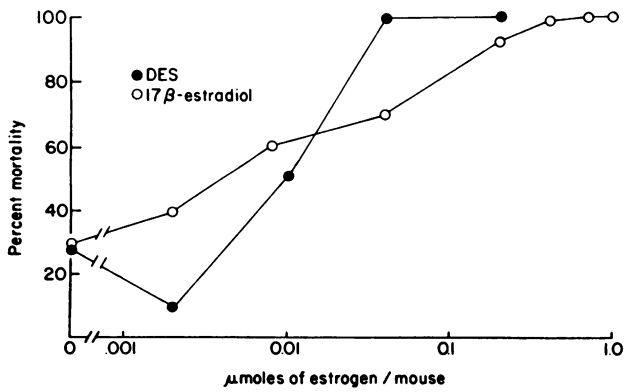

FIG. 1. Effect of DES and 17β-estradiol exposure on survival of B6C3F$_1$ female mice after *Listeria* infection. Each point represents a minimum of 10 animals. Significantly increased mortality ($P < 0.05$) occurred in mice treated with more than 0.04 μmol of DES or estradiol.

TABLE 1. Susceptibility to *Listeria* cells after exposure to compounds with various estrogenic activities[a]

Treatment[b]	No. dead/ no. tested	% Mortality
Vehicle (control)	23/82	28
DES	51/57	90[c]
Estradiol	24/27	89[c]
5α-DHT	2/10	20
Progesterone	3/15	20
Zearalenol	4/27	15
α-Dienestrol	12/15	80[c]
β-Dienestrol	0/15	0
Corticosterone	1/15	7

[a] Data represent a summation of several experiments in which vehicle-and DES-or estradiol-exposed mice were used as controls. *Listeria*-related mortality increased significantly as a result of exposure to either DES or estradiol in each experiment. The mortality range averaged from 20 to 25% for each group.

[b] Mice received a total dose of 2.8 μmol of chemical (which equals 40 mg of estradiol per kg of body weight).

[c] Significantly different from vehicle controls at $P < 0.05$.

macrophages and to break up clumps of bacteria. Duplicate plates were prepared for all samples at each dilution. Reported *Listeria* titers represent mean values obtained from six individual mice per group.

In vivo listericidal assay. Mice were injected intraperitoneally (i.p.) with 5×10^5 *Listeria* in 0.5 ml of Hanks balanced salt solution. At indicated times after infection, bacteria were obtained from the peritoneal cavity by lavage with 5 ml of Hanks balanced salt solution. Serial dilutions in Hanks balanced salt solution plus 0.05% Triton X-100 were prepared and plated in duplicate on brain heart infusion agar to determine the number of viable *Listeria* cells. Cytocentrifuge preparations of peritoneal exudate cells were stained with Diff-Quik (American Scientific Products, McGaw Park, Ill.) and used to calculate differential cell counts. The total number of peritoneal exudate cells was determined with a Coulter Counter (model ZB; Coulter Electronics, Inc., Hialeah, Fla.). Reported *Listeria* titers and peritoneal exudate cell numbers represent mean values obtained from five individual mice per group.

PYB6 tumors. A polyoma virus-induced tumor developed in C57BL6 mice (PYB6) was obtained from Jack Dean (Chemical Industry Institute of Toxicology, Research Triangle Park, N.C.) and maintained by passage through syngeneic B6C3F$_1$ female mice. For experimental studies, mice were inoculated subcutaneously in the right rear flank with 3×10^3 viable tumor cells (10 to 20% tumor dose) suspended in RPMI 1640 medium containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer plus 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) 4 to 5 days after estrogen exposure. Animals were palpated weekly for 60 days, and tumor frequency, size, and latency were recorded.

Statistical analysis. The chi-square test was used to assess the significance of treatment effects in mortality studies. Student's *t* test was used to compare results obtained from experimental groups in all other instances.

RESULTS

The administration of either estradiol or DES resulted in a marked increase in the susceptibility of female B6C3F$_1$ mice to *L. monocytogenes*. Significantly increased mortality after *Listeria* infection occurred in mice treated with greater than 0.04 μmol of DES or estradiol (Fig. 1). Time to death

decreased from 7 days in control mice to ca. 5 days ($P < 0.001$) in mice exposed to 2.8 μmol of either compound (data not shown). Table 1 summarizes the results obtained after exposure of mice to equimolar concentrations (2.8 μmol) of various hormonally active compounds before *Listeria* infection. DES, estradiol, and α-dienestrol, which display relatively high estrogenic activity, caused a significant increase in mortality. Compounds such as 5α-DHT, progesterone, zearalenol, and β-dienestrol, which have little or no estrogenic activity, did not increase mortality. Corticosterone was also found to have no effect on *Listeria* resistance at this dose level.

To determine if increased *Listeria* susceptibility after estrogen treatment was due to an ovarian dysfunction induced by estrogen treatment, adult mice were ovariectomized and exposed to estradiol before *Listeria* infection. Ovariectomy did not influence the effects of exposure to pharmacological levels of estrogen on resistance as both sham-operated and ovariectomized mice were equally susceptible to *L. monocytogenes* after estradiol treatment (Table 2).

The influence of antiestrogens on host resistance was examined by simultaneously administering estradiol and tamoxifen, a nonsteroidal estrogen antagonist. The increase in host susceptibility due to estradiol was prevented by tamoxifen treatment (Table 3). *Listeria*-related mortality in mice dosed with tamoxifen plus estradiol was similar to that of vehicle controls. Exposure to tamoxifen alone did not affect survival.

Since it has been proposed that estrogens influence immune functions by altering thymic hormone levels (9, 24, 42), we examined the effect of surgical thymectomy (TX) on estrogen-induced alterations in host resistance. In these studies, adult female mice were thymectomized and treated with estradiol before *L. monocytogenes* infection. TX afforded protection against estrogen-altered host resistance after low-dose estrogen administration (Table 4). *Listeria*-related mortality in thymectomized mice dosed with 0.04 μmol of estradiol was similar to that observed in vehicle controls. On the other hand, estradiol-exposed sham-operated mice had significantly higher mortality than did controls. The protective effect of TX on *Listeria* resistance was not observed in mice treated with a greater dose of estradiol (0.74 μmol), which suggests that at higher exposure levels, nonthymic mechanisms may be involved. Intermediate dosage levels were not examined. A similar experiment was performed with transplantable tumor cell inoculation rather than *Listeria* infection to reconfirm that the thymus plays a role in estrogen-induced alterations of host resistance (Table 4). TX protected mice against the effects of estrogen on syngeneic tumor formation. Tumor incidence in both DES-exposed and control thymectomized mice was 50%. Tumor incidence in sham-operated mice treated with DES (90%) was significantly higher than in controls.

TABLE 2. Effects of 17β-estradiol on susceptibility to *Listeria* cells after adult ovariectomy (OVX)

Total dose of estradiol (μmol)	Intact (sham)		OVX	
	No. dead/ no. tested	% Mortality	No. dead/ no. tested	% Mortality
0 (vehicle)	2/13	15	4/13	31
0.04	6/14	43	5/14	36
0.40	8/14	57[a]	11/14	78[a]

[a] Significantly different from vehicle controls at $P < 0.05$.

TABLE 3. Susceptibility to *Listeria* cells after exposure to estradiol and tamoxifen

Treatment[a]	No. dead/ no. tested	% Mortality
Vehicle	1/15	7
Tamoxifen	2/15	13
Estradiol	14/15	93[b]
Tamoxifen + estradiol	2/15	13

[a] Mice were dosed with a total of 3.6 μmol of tamoxifen or 2.4 μmol of estradiol or both.
[b] Significantly different from vehicle controls at $P < 0.05$.

The growth kinetics of *L. monocytogenes* in the spleens of estrogen-treated mice was determined by monitoring splenic bacterial growth over a 7-day period after i.v. inoculation with a sublethal dose of *L. monocytogenes* (Fig. 2). One day after infection no differences were observed between the numbers of *Listeria* cells in the spleens of control and estrogen-treated mice. However, there were significantly higher numbers of bacteria in the spleens of both DES and estradiol-treated mice when compared with control mice on days 4 and 7 after infection. Estrogen exposure had the same effect on *Listeria* titers in the livers of i.v.-infected mice and the spleens of i.p.-infected mice (data not shown).

To examine the effect of estrogen exposure on leukocyte accumulation at infective foci, we determined the number of peritoneal lymphocytes, monocytes, and polymorphonuclear leukocytes (PMNs) after an i.p. injection of viable *Listeria* cells. At the same time the peritoneal growth kinetics of the bacteria were monitored. Estrogen exposure depressed the accumulation of lymphocytes and monocytes in the peritoneal cavities of i.p.-infected mice (Fig. 3). The number of peritoneal lymphocytes and monocytes was greater in mice exposed to DES than in vehicle controls on the day of infection. However, the quantity of lymphocytes rose to significantly higher levels in the controls by day 4 after infection and remained elevated above peritoneal lymphocyte numbers in the DES-treated mice through day 5. The number of peritoneal monocytes increased to significantly higher levels in the controls than in the DES-treated mice on days 3 and 4 after infection. Comparable quantities of monocytes were not observed in the DES-exposed animals until day 5. DES treatment augmented rather than delayed the accumulation of PMNs in the peritoneal cavities of i.p.-infected mice. Peritoneal accumulation of PMNs rose to peak levels on day 2 in the controls and then declined. The number of PMNs in the peritoneal cavities of the DES-treated mice had risen to significantly higher levels than observed in the controls by day 3 and remained significantly elevated on days 4 and 5.

TABLE 4. Effect of estrogen on susceptibility to *Listeria* and PYB6 tumor cells after adult TX

Total dose of estrogen[a] (μmol)	No. dead/no. tested (%) with *Listeria* cells		Tumor incidence/no. tested (%) with PYB6 tumor cells	
	Intact (sham)	TX	Intact (sham)	TX
0 (vehicle)	4/19 (21)	7/27 (26)	4/10 (40)	5/10 (50)
0.04	13/15 (87)[b]	11/29 (38)	ND[c]	ND
0.74	20/24 (75)[b]	18/24 (75)[b]	9/10 (90)[b]	5/10 (50)

[a] Mice were treated with estradiol in the *Listeria* studies and with DES in the tumor study.
[b] Significantly different from vehicle controls at $P < 0.05$.
[c] ND, Not done.

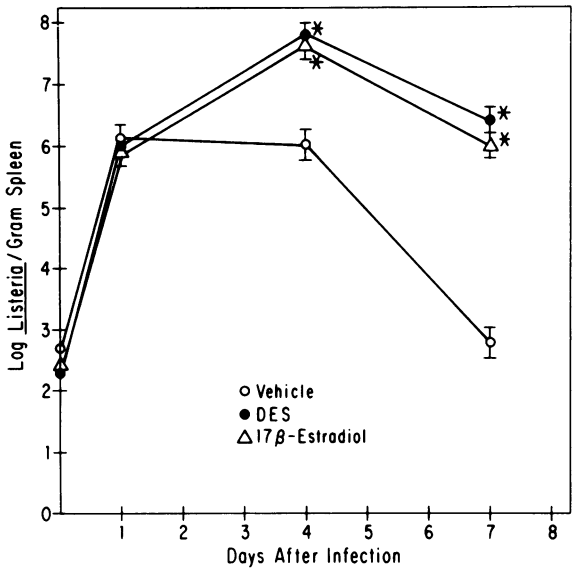

FIG. 2. Growth of *Listeria* cells in the spleens of mice treated with vehicle, 2.8 μmol of DES, or 2.8 μmol of estradiol. Each point represents the mean value ± 1 standard error from six mice. *, Significantly different from vehicle controls at $P < 0.05$.

There was a slight, though significant, increase in the number of bacteria in the peritoneal cavities of the DES-treated animals 1 day after i.p. infection (Fig. 4). On subsequent days the number of bacteria remained lower in the control mice than in the DES-exposed mice and declined to essentially nonexistent quantities in the controls by day 5. The number of bacteria in the DES-treated mice, on the other hand, remained ca. 3 logs higher than in the controls from day 3 through day 5. We did not observe measurable quantities of *Listeria* cells in the peritoneal cavities of either vehicle- or DES-treated mice after i.v. infection.

DISCUSSION

These studies demonstrate that pharmacological (i.e., therapeutic) levels of steroidal and nonsteroidal estrogens profoundly alter the susceptibility of mice to *L. monocytogenes* infection. The ability of estrogenic compounds to alter host resistance to *Listeria* cells appears to correlate with their estrogenicity. The classical measure of estrogenic activity, uterine growth, is initiated after hormone binding to cytoplasmic receptors and subsequent translocation of the hormone-receptor complex into the nucleus where binding to the nuclear chromatin occurs (10, 20). DES and 17β-estradiol have similar in vitro uterotrophic potency and binding activity although DES is more potent than estradiol in vivo (19). DES, 17β-estradiol, and α-dienestrol possess in common a phenolic ring structure in the A-ring region of the molecule which is believed to be responsible for specific binding to estrogen receptors (6). Administration of pharmacological levels of these compounds caused increased mortality after *Listeria* infection. Increased mortality also occurred in estrogen-treated ovariectomized mice; thus, the effects of estrogenic compounds on *Listeria* resistance are not indirectly mediated through ovarian dysfunction. Steroidal and nonsteroidal compounds possessing little or no estrogenic activity did not alter *Listeria* resistance. These compounds included progesterone, 5α-DHT, β-dienestrol, and zearalenol. β-Dienestrol, although not estrogenic, is a potent genotoxic metabolite of DES capable of binding to macromolecules and potentially a direct carcinogen (37). 5α-DHT is a hormonally active metabolite of testosterone which demonstrates slight uterine binding activity but only at relatively high doses (40). Zearalenol possesses 20% of the receptor-binding activity of estradiol and only 1% of its uterotrophic activity (16), suggesting that binding alone will not induce altered resistance. Exposure to 2.8 μmol of corticosterone also had no effect on *Listeria*-related mortality. Cortisone acetate has been reported to affect *Listeria* resistance, but at dosage levels higher than used in our experiments (34, 39). In contrast, exposure to as little as 0.04 μmol of DES or estradiol significantly increased mortality.

A direct relationship between estrogenic activity and increased susceptibility to *L. monocytogenes* was also evidenced in studies with the estrogen antagonist tamoxifen. This compound is a nonsteroidal derivative of triphenylethylene, has only limited estrogenic activity, and putatively acts as an antiestrogen by inhibiting the replenishment of cytoplasmic estrogen receptors (43). We observed that the effects of estradiol on *Listeria* resistance are blocked by concurrent administration of tamoxifen. This further supports the idea that estrogen-induced modifications of *Listeria* resistance are, at least in part, due to estrogenic activity and thus mediated through an operational receptor-like mechanism similar to the cytosolic estrogen receptor involved in uterotrophic responses.

The reported immunological effects associated with the administration of estradiol and DES to rodents include thymic atrophy (5), myelotoxicity (1, 7), stimulation of the mononuclear phagocyte system (1, 22, 31, 49), suppression of cell-mediated immune responses (12, 13, 23), and depres-

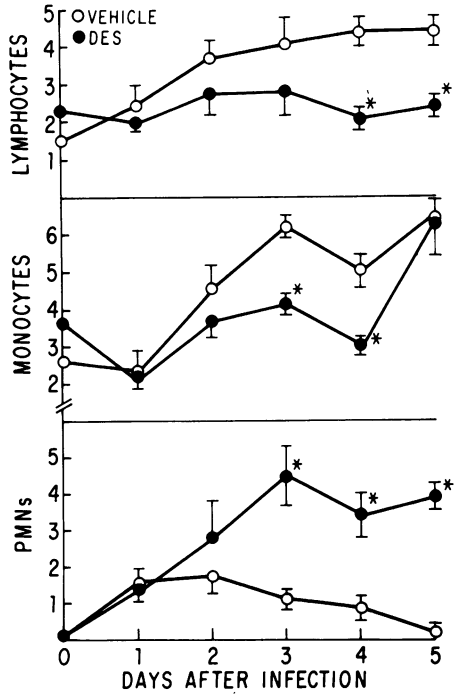

FIG. 3. Number of peritoneal lymphocytes, monocytes, and PMNs ($\times 10^6$) in vehicle control and DES (2.8 μmol)-treated mice after i.p. *Listeria* infection. Each point represents the mean value ± 1 standard error from five mice. *, Significantly different from vehicle controls at $P < 0.05$.

sion of natural killer cell activity (14, 41). The mechanisms responsible for the wide range of effects of estrogens on immunological responses are not well understood, although increasing evidence suggests that estrogen-dependent immunotoxicity is, in part, mediated through the thymus. For example, experimental evidence suggests that the binding of estrogen to specific cytosolic receptors in thymic epithelial cells influences the quality and quantity of thymic hormones (9, 24, 42). That this may represent a negative regulatory function was suggested in studies in which surgical TX was found to protect against estrogen-induced immunosuppression (24). In the present study, we observed that TX was protective against estrogen-induced increased susceptibility to *L. monocytogenes*. Thus, certain of the effects of estrogens on host resistance are related to alterations in thymic immunoregulation. We noted, however, that when the exposure level of estradiol was increased, the protective effect of TX was lost. These findings may be reflective of a dual mode of action of estrogens: one which is mediated by the thymus at low-dosage levels and a second which directly affects immune functions at high-dosage levels. This is consistent with the proposed mechanisms responsible for changes in immune function by estrogens (M. I. Luster, R. W. Pfeifer, and A. N. Tucker, *in* J. Dean, A. E. Munson, M. I. Luster, and H. Amos [ed.], *Target Organ Toxicity*, in press).

In a similar experiment, transplantable syngeneic tumor cell growth was used as the infectivity model. The resistance of mice to syngeneic tumor cells is primarily dependent on macrophage- and T cell-mediated immunity (45) and has been previously shown by us to be decreased by estrogen exposure (4). In the present study, we demonstrated that the effects of estrogen on resistance to syngeneic tumor cells could be inhibited by adult TX. This further confirms that the thymus plays a role in estrogen-induced modulation of host resistance.

The ability of mice to resist *Listeria* infection primarily depends on the cell-mediated immune response. Recovery is thought to require T-cell activation of the bactericidal properties of macrophages (21, 25, 35) and the accumulation of large numbers of macrophages at infective foci (28, 33, 34, 38). Since estrogen exposure clearly alters macrophage and T-cell functions, experiments were performed to determine the role of these alterations on the ensuing *Listeria* infection.

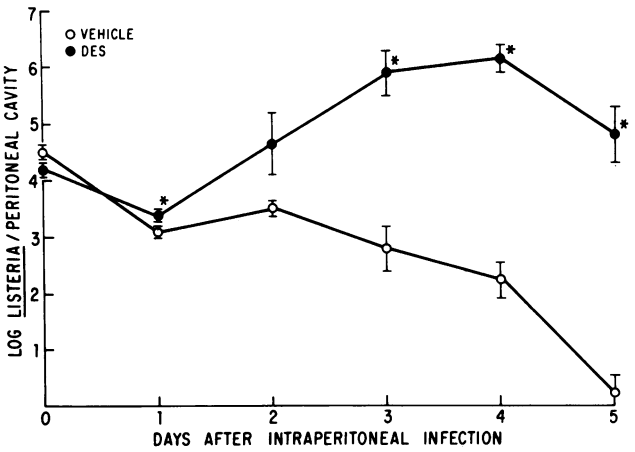

FIG. 4. Growth of *Listeria* cells in the peritoneal cavities of vehicle control and DES (2.8 μmol)-treated mice after i.p. infection. Each point represents the mean value ± 1 standard error from five mice. *, Significantly different from vehicle controls at $P < 0.05$.

The effects of estrogens on T cells are multifold. For example, estrogen exposure induces thymic involution (1, 5) and depresses delayed hypersensitivity as well as lymphoproliferative responses to allogeneic leukocytes and T-cell mitogens (13, 23). Estrogens may also depress interleukin-2 production (11). Indirect evidence that estrogens disrupt the T cell-mediated immune response to *Listeria* cells was provided by following the growth of bacteria in the spleens of estrogen-exposed animals. Substances which are directly toxic to macrophages are presumably evidenced by greater than normal numbers of bacteria in the liver and spleen as early as 24 h after infection. T cell-mediated defects, on the other hand, are evidenced by greater than normal numbers of bacteria in these organs only later in the infection, when complete elimination of the bacteria is thought to be dependent on T cell-dependent events such as macrophage activation (48). As was shown in this study and suggested by preceding studies in this laboratory (4), the number of bacteria in the spleens of estrogen-treated mice was greater than in control mice only later in the infection. This may indicate that the ability of nonimmune macrophages to ingest and destroy *Listeria* cells is not affected by estrogen exposure but that the disruptive effects of estrogens on *Listeria* resistance are related to altered T-cell functions.

The known effects of estrogen exposure on the mononuclear phagocyte system include augmented antigen clearance by the liver (22, 31) and increased peritoneal macrophage activity (1). Peritoneal macrophages from estrogen-treated mice do not appear to be fully activated but possess certain characteristics of inflammatory macrophages. They are hyperphagocytic, inhibit tumor cell growth in vitro, and secrete increased quantities of plasminogen activator (1; J. H. Dean, M. I. Luster, B. Adkins, L. D. Lauer, and D. O. Adams, *in* A. Volkman [ed.], *Mononuclear Phagocyte Biology*, in press). Their bactericidal properties are unknown. We observed that the number of bacteria in the peritoneal cavities of DES-treated mice was only slightly elevated above that of the control mice 24 h after an i.p. infection, despite similar numbers of peritoneal macrophages in both control and treated mice at that time. Similarly, there was no difference between splenic titers of *Listeria* cells in either group 24 h after an i.v. infection. This may indicate that the ability of nonimmune macrophages to kill *Listeria* cells is neither augmented nor depressed by estrogen exposure. We are presently investigating this possibility by studying the in vitro bactericidal properties of these cells. An estrogen-induced enhancement of the early in vivo bactericidal capacity of fixed liver macrophages has been reported (29). However, estrogen exposure may increase the number of liver macrophages as well as their activity (50). Thus, augmented early destruction of *Listeria* cells in the livers of estrogen-exposed mice could be due to increased macrophage numbers rather than, or in addition to, an enhancement of the bactericidal capacity of individual cells.

Later in the infection, when macrophage accumulation and activation are thought be be responsible for elimination of the bacteria (25, 28, 33, 34), *Listeria* titers in infective foci of DES-exposed mice were greatly elevated above control levels. The fulminant growth of *Listeria* cells which occurred in the peritoneal cavities of DES-exposed mice after an i.p. infection was accompanied by a delay in the accumulation of macrophages and lymphocytes at the site of infection. The inability of DES-exposed mice to increase the number of macrophages in infective foci at the same rate as controls may account for the virtually unrestricted growth of the bacteria in these animals. The delay in mononuclear cell

accumulation could be due to a variety of different factors. One possible explanation relates to the known effects of estrogens on lymphokine production. Estrogen exposure reportedly decreases interleukin-2 production (11). This might indirectly affect macrophage accumulation at infective foci by depressing the proliferation of T cells responsible for the production of other lymphokines including macrophage chemotactic factor and migration inhibition factor.

Estrogens do not reduce PMN migration into infective foci. Large numbers of PMNs can be observed in the peritoneal cavities of DES-exposed mice up to 5 days after an i.p. infection. Multiplication of bacteria was virtually unrestricted in the estrogen-exposed mice despite the quantity of PMNs present in these animals. Although this may confirm earlier reports that PMNs play little role in *Listeria* resistance in mice (34, 44), there is now evidence that they can kill *Listeria* cells in vitro (3). Consequently, an estrogen-induced defect in the ability of these cells to destroy the bacteria cannot be ruled out. There is no obvious explanation for sustained PMN accumulation at infective foci after DES exposure. Other anti-inflammatory agents, most notably the glucocorticosteroids, may induce similar effects. Cortisone acetate, for example, augments PMN accumulation in the livers of *Listeria*-infected mice (34). Dexamethasone enhances PMN migration in vitro but has the opposite effect on macrophages (46).

In summary, estrogen-induced suppression of *Listeria* immunity may be mediated by an operational hormone receptor mechanism and is partially regulated through the thymus. The effects of estrogen on host defenses include a depression in the ability of exposed mice to accumulate monocytes and lymphocytes or eliminate bacteria at infective foci. These effects may be related to an estrogen-induced suppression of the T cell-dependent immune response. These findings are of relevance in view of the fact that estrogenic compounds such as DES and estradiol are commonly used for both therapeutic and agricultural purposes (27, 32). Clinical observations have provided evidence that the therapeutic use of estrogens can induce immunotoxicity (Luster et al., *in* Dean et al. (ed.), *Target Organ Toxicity*, in press), and the apparent ability of these compounds to affect susceptibility to infectious diseases should be considered an additional health hazard.

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