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Influence of Estrogen on Host Resistance: Increased Susceptibility of Mice to *Listeria monocytogenes* Correlates with Depressed Production of Interleukin 2

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Mice given pharmacological levels of the synthetic estrogen diethylstilbestrol demonstrated a marked increase in susceptibility to infection with *Listeria monocytogenes*. Experiments were performed in an effort to determine the mechanism(s) by which estrogen treatment increases the susceptibility of mice to *L. monocytogenes* infection. Estrogen exposure depressed the in vivo proliferative response of splenic lymphocytes to *L. monocytogenes*, which correlated with the decreased in vitro response of these cells to phytohemagglutinin. Interleukin 2 (IL 2) production by splenic lymphocytes from estrogen-treated mice was decreased, although these cells were capable of proliferating normally in response to exogenous IL 2. Interleukin 1 production by peritoneal macrophages was not depressed by estrogen exposure. The number of bacteria observed in the spleens of estrogen-exposed mice challenged with *L. monocytogenes* was reduced by IL 2 administration. Thus, estrogens may decrease host resistance to *L. monocytogenes* by inhibiting IL 2 production and the subsequent proliferation of antigen-sensitized T lymphocytes required for recovery.

Estrogenic compounds are commonly encountered in the environment. For example, diethylstilbestrol (DES), a synthetic nonsteroidal estrogen, was extensively used as a growth-promoting agent in the sheep and cattle industries (reviewed in reference 26). In addition, DES has been widely used in humans as an antiabortive agent, to control menopausal symptoms, to suppress lactation, to prevent postcoital implantation, and to treat breast cancer and prostate cancer (4, 31). The toxicological effects associated with the therapeutic use of DES include mixed adenosquamous carcinomas of the uterus (4) and clear-cell adenocarcinomas of the vagina in young women exposed in utero (11). In addition, immunosuppression after exposure to therapeutic-dose levels of estrogen has been demonstrated in humans (reviewed in reference 14) and animals (reviewed in reference 25).

Observations of sex differences in host resistance to infectious agents have prompted numerous studies dealing with the effects of estrogens on infectious disease processes (e.g., 5, 7, 29, 30, 35, 37). These studies have clearly indicated that therapeutic and, in some instances physiological, levels of estrogens alter host susceptibility to selected agents. Although the underlying mechanisms are not well understood, such phenomena presumably reflect estrogen-induced immunosuppression. In this respect, it has been suggested that estrogens modulate T-cell maturation by interfering with normal thymic hormone production (9, 24, 27). Previous work in this laboratory indicated that decreased host resistance to infectious agents, particularly *Listeria monocytogenes*, is one of the most sensitive pathological indicators of altered estrogen levels in mice (34). Increased susceptibility to *L. monocytogenes* infection occurred in mice exposed to DES or 17β-estradiol at dose levels as low as 0.04 μmol (34). Furthermore, in studies with ovariectomized mice restored with estradiol 17β-cypionate it was noted that although physiological levels of estrogens did not influence resistance to either *L. monocytogenes* or *Toxoplasma gondii*, abnormal physiological levels, like those that occur during pregnancy, increased susceptibility (O. J. Pung and M. I. Luster, Exp. Parasitol., in press). Earlier, Luft and Remington (21) reported that pregnant mice are more susceptible to both of these organisms.

The present studies were performed to investigate in detail the relationship between estrogen-induced immunosuppression and altered host susceptibility to *L. monocytogenes*. The in vitro and in vivo effects of estrogen on lymphoblastogenesis were investigated, as were the roles of interleukin 1 (IL 1) and interleukin 2 (IL 2) in altered host resistance.

MATERIALS AND METHODS

Mice. Specific-pathogen-free B6C3F$_1$ (C57BL/6 × C3H/He) female mice were obtained under a National Cancer Institute production contract from Charles River Breeding Laboratories, Inc., Portage, Mich. Animals were 8 to 10 weeks of age and weighed an average of approximately 20 g at the time of these studies. They were maintained on a 12-h light-dark cycle at 20 to 23°C and were provided with water and autoclaved food (Ziegler Brothers, Inc., Gardner, Pa.) ad libitum.

Bacterium. *L. monocytogenes* L242/73 type 4B from a naturally infected mouse was stored at −70°C in nutrient broth plus 15% glycerol. For infection of mice, a sample of the frozen stock was thawed and diluted in Hanks balanced salt solution, and 10^4 CFU were injected intravenously via the tail vein.

Chemical-exposure regimen. Mice were treated with DES (Sigma Chemical Co., St. Louis, Mo.) daily for five consecutive days. The chemical was dissolved in corn oil with gentle heating and diluted such that each mouse received a daily volume of 0.1 ml of corn oil subcutaneously in the

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dorsal cervical region. Controls received an equal volume of corn oil. Studies were begun 3 to 5 days after the last dose.

Preparation of lymphoid cell suspensions. The culture medium used in these experiments, henceforth referred to as complete medium (CM), consisted of RPMI 1640 culture medium containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, 2 mM L-glutamine (GIBCO Laboratories, Grand Island, N.Y.), and 50 μg of gentamicin sulfate (Sigma) per ml. CM was supplemented with fetal calf serum (FCS; GIBCO) or human AB serum as indicated below. Single cell suspensions of spleens or thymus glands were prepared by mashing the organ in 1 ml of CM supplemented with 10% FCS with the plunger end of a syringe. The cells were passed through a 25-gauge needle into a 4-ml culture tube. Clumped cells were allowed to settle for 2 min, and 0.2 ml of the supernatant fluid containing suspended cells was placed in 2 ml of fresh CM. Nucleated cells were enumerated with a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.), and their viability was always >95%, as assessed by trypan blue exclusion.

Cell identification. Cytocentrifuged preparations stained with Diff-Quik (American Scientific Products, McGaw Park, Ill.) were used to determine the percentages of lymphocytes, macrophages, and polymorphonuclear leukocytes. B and T cells were enumerated by fluorescence staining. Briefly, gradient-separated spleen cells (Lympholyte M; Accurate Chemical and Scientific Corp., Westbury, N.Y.) were washed three times in phosphate-buffered saline containing 5% FCS and adjusted to a concentration of 2×10^7 cells per ml. Samples (0.05 ml) of cells were placed in microconical tubes, and 0.05 ml of a 1/10 dilution of fluorescein isothiocyanate-conjugated anti-Thy 1.2 or rabbit antimouse immunoglobulin (Litton Bionetics, Kensington, Md.) was added. After incubation for 30 min at 23°C, the cells were washed, the supernatant fluid was decanted, and the cells were mixed with 0.1 ml of phosphate-buffered saline containing 10% glycerol. A total of 200 cells per sample were counted with a Leitz fluorescence microscope.

Mitogen assay. The ability of lymphocytes to respond to mitogens was determined by a microculture assay as previously described (22). Purified phytohemagglutinin (PHA; Burroughs Wellcome Co., Research Triangle Park, N.C.) and *Escherichia coli* lipopolysaccharide (LPS; serotype O111:B4; Sigma) were used as stimulators at various concentrations. At 6 h before culture termination, 1.0 μCi of tritiated thymidine ([^{3}H]TdR; New England Nuclear Corp., Boston, Mass.) was added to each well. The cells were harvested, and [^{3}H]TdR incorporation was determined by liquid scintillation spectroscopy.

IL 1. IL 1 was produced and quantitated by a modification of the method of Meltzer and Oppenheim (27). Peritoneal exudate cells were obtained by peritoneal lavage of mice with 10 ml of Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution containing 10 U of sodium heparin per ml. The cells were centrifuged for 10 min at 500 × *g* and adjusted to a concentration of 10^6/ml in CM containing 5% FCS (HyClone; Sterile Systems, Logan, Utah) and 2.5×10^{-5} M 2-mercaptoethanol (2-ME). Aliquots (7 ml) of suspended cells were incubated in 25-cm^2 tissue culture flasks at 37°C in a humidified atmosphere containing 5% CO_2. After 2 h, the flasks were rinsed three times with CM to remove nonadherent cells and refilled with 7 ml of CM containing 5% FCS, 2.5×10^{-5} M 2-ME, and 50 μg of LPS per ml. After 2 days of incubation, the supernatants were collected, dialyzed against CM, filter sterilized, and frozen at −20°C. For quantitation of IL 1 in these preparations, pooled thymocytes were added to triplicate wells of flat-bottom, 96-well microtiter plates in 0.05-ml volumes (1.5×10^6 cells) in CM containing 5% human AB serum and 2.5×10^{-5} M 2-ME. IL 1 preparations were diluted in this medium and added to the wells in 0.1-ml volumes to produce final IL 1 dilutions of 1:2, 1:4, and 1:8. PHA in CM was then added in 0.05-ml volumes to yield a final concentration of 2 μg/ml. Plates were incubated for 72 h at 37°C in 5% CO_2–air and pulsed with 1 μCi of [^{3}H]TdR 18 h before harvest.

IL 2. For some experiments, IL 2 was produced by a modified version of the technique of Hoffenbach et al. (12). Mouse spleen cells, adjusted to a concentration of 5×10^6/ml in CM containing 5% human AB serum, 5×10^{-5} M 2-ME, and 2 μg of conconavalin A (ConA; Sigma) per ml, were added in 7-ml volumes to 25-cm^2 tissue culture flasks. The cells were incubated for 24 h, after which the supernatant were collected, treated with 0.1 M α-methyl-D-mannoside (Sigma), filter sterilized, and frozen at −20°C. Human recombinant IL 2 (Genzyme Corp., Boston, Mass.) was used as a standard and for in vivo restoration experiments. In the restoration experiments, mice were inoculated intraperitoneally with 1,500 U of recombinant IL 2 in 0.5 ml of saline on the day of *L. monocytogenes* infection; control mice received saline.

IL 2 preparations were tested against ConA blast cells as follows. Aliquots (2 ml) of spleen cells (10^6/ml) were incubated for 72 h in CM containing 5% human AB serum, 5×10^{-5} M 2-ME, and 2 μg of ConA per ml. After incubation, the cells were washed twice in CM containing 5% human AB serum and 0.1 M α-methyl-D-mannoside, and their viability was determined by trypan blue exclusion. Cells in CM containing 5% human AB serum and 5×10^{-5} M 2-ME were added to triplicate wells of flat-bottom microtiter plates in 0.1-ml volumes (3×10^4 cells). IL 2 preparations were diluted in this medium and added to the wells in 0.1-ml volumes. The cells were cultured for 72 h and pulsed with 1.0 μCi of [^{3}H]TdR 6 h before harvest.

Statistical analysis. The chi-square test was used to assess the significance of treatment effects in *L. monocytogenes* mortality studies. For single comparisons of means, Student's *t* test was used. For multiple comparisons of means, significance levels were determined by a one-way analysis of variance (ANOVA), and means were compared by the least-significant-difference (LSD) test (40).

RESULTS

The total number of nucleated spleen cells in estrogen-exposed and control mice was quantitated at various times after *L. monocytogenes* infection to determine the effects of estrogen on leucocyte accumulation at infective foci (Fig. 1). The total number of nucleated spleen cells in uninfected controls remained at approximately the same level throughout the course of the experiment. Total spleen cell numbers in both infected and uninfected DES-exposed mice, although initially higher than that in uninfected controls, progressively declined throughout the experiment. In contrast, spleen cell numbers in infected controls increased to levels significantly higher than those observed in both uninfected controls and infected DES-exposed mice by day 4 after infection and rose to even higher levels by day 6. This increase in spleen cell numbers was primarily due to an increase in the number of immunoglobulin- and Thy 1.2-positive lymphocytes (Table 1). By day 6 after infection, immunoglobulin- and Thy 1.2-positive spleen cell numbers were significantly higher in infected controls than in either uninfected controls or infected DES-exposed mice. Macro-

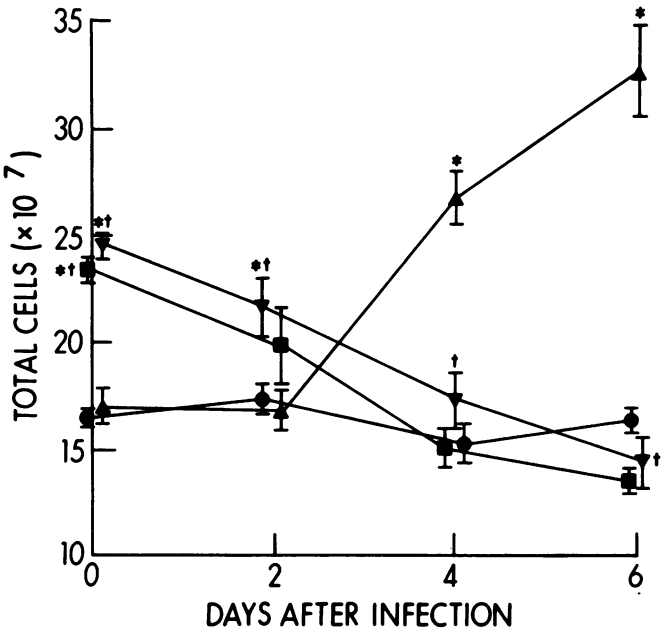

FIG. 1. Effect of DES or *L. monocytogenes* infection or both on total nucleated spleen cell numbers in uninfected mice given corn oil (●) or 3 μmol of DES (■) or in infected mice given corn oil (▲) or 3 μmol of DES (▼). Mice were inoculated intravenously with 10^4 *L. monocytogenes* cells 4 days after the final chemical treatment. Each value represents the mean ± 1 standard error for at least eight mice. Asterisks indicate significantly differences from uninfected controls ($P < 0.05$). Daggers indicate significant differences from infected mice given corn oil ($P < 0.05$).

phage numbers were also significantly higher in infected controls on day 6, whereas the number of polymorphonuclear leukocytes was higher in infected DES-exposed mice.

A series of experiments was performed to determine the mechanism by which estrogens suppress lymphocyte accumulation and proliferation at infective foci. Spleen cells from estrogen-exposed mice were cultured with mitogens to quantitate their proliferative capacity. As has previously been demonstrated (22, 24), exposure to 3.0 μmol of DES significantly depresses the ability of splenic lymphocytes to respond to the T-cell mitogen PHA and the B-cell mitogen LPS (data not shown). The effect of DES exposure on the production of IL 2 was examined by obtaining supernatant containing IL 2 from ConA-incubated cultures of splenic lymphocytes of DES-treated or control mice. IL 2 production was quantitated by measuring the ability of the supernatant to support ConA blast cell proliferation. It was observed that IL 2 supernatant produced from lymphocytes of DES-exposed mice did not stimulate ConA blast cell proliferation to the same degree as IL 2 supernatant from control lymphocytes and that this decrease in IL 2 production was dose dependent (Table 2). Blast cell proliferation in the presence of a 1:2 dilution of IL 2 supernatant fluid produced from lymphocytes of mice exposed to 0.07, 0.74 or 3.0 μmol of DES was approximately 37, 30, and 13%, respectively, of the control values.

TABLE 2. Effect of DES exposure on the ability of spleen cells from $B6C3F_1$ female mice to produce IL 2

Total amt of DES injected (μmol)[a]	[^{3}H]TdR incorporated (cpm × 10^3)[b] by ConA blast cells at indicated dilution of IL 2 supernatant	
	1:2	1:4
0	22.7 ± 1.7	13.7 ± 2.2
0.07	8.5 ± 1.7 (37)[c]	3.0 ± 0.7 (22)[c]
0.74	7.0 ± 0.9 (30)[c]	2.1 ± 0.3 (15)[c]
3.00	4.3 ± 0.7 (13)[c]	2.4 ± 0.3 (17)[c]

[a] Mice were treated with DES for 5 days. Controls received corn oil.
[b] Three days after the final treatment with DES or corn oil, spleen cells from exposed or control mice were cultured with 2 μg of ConA per ml to produce IL 2 supernatants. Supernatants were tested for the presence of IL 2 by measuring their ability to stimulate the proliferation of blast cells prepared from the spleens of unexposed mice. Each value represents the mean ± 1 standard error for two sets of pooled spleen cells used to produce IL 2 supernatants (three mice per pool). Numbers in parentheses represent percentages relative to control mice. The value for medium-treated controls was 2.4 cpm × 10^3.
[c] Significantly different from controls ($P < 0.05$) as determined by Student's *t* test.

The effect of DES exposure on the response of lymphocytes to IL 2 was examined by culturing ConA blast cells produced from splenic lymphocytes of mice exposed to 3.0 μmol of DES in the presence of IL 2. The proliferative response of ConA blast cells prepared from the spleens of DES-exposed mice was only slightly lower (not significantly) than that of the control blast cells (Table 3).

To directly examine the effect of IL 2 on the estrogen-mediated increase in susceptibility of mice to *L. monocytogenes*, we inoculated DES-exposed mice intraperitoneally with 1,500 U of human recombinant IL 2 on the day of *L. monocytogenes* infection. Four days later, the number of splenic bacteria was determined. IL 2 partially restored host resistance to *L. monocytogenes* in DES-exposed mice (Fig. 2). Although the number of bacteria observed in the spleens of DES-treated and DES-treated, IL 2-treated mice was significantly higher than the number observed in corn oil controls, bacterial counts in the spleens of mice treated with

TABLE 1. Effect of DES exposure on differential spleen cell counts 6 days after *L. monocytogenes* infection

Mice[a]	No. of cells per spleen (10^7)[b]				
	Total	Immunoglobulin positive	Thy 1.2 positive	Macrophages	Polymorphonuclear leukocytes
Uninfected control	16.6 ± 0.8	5.4 ± 0.8	5.4 ± 0.5	0.4 ± 0.1	0.3 ± 0.1
Uninfected DES exposed	14.4 ± 1.0	5.1 ± 0.5	5.1 ± 0.4	0.4 ± 0.1	0.3 ± 0.1
Infected control	31.0 ± 2.8[c]	10.3 ± 1.3[c]	13.2 ± 2.0[c]	2.2 ± 0.3[c]	1.3 ± 0.2
Infected DES exposed	15.9 ± 1.2[d]	5.6 ± 0.7[d]	5.6 ± 0.7[d]	1.2 ± 0.2	2.6 ± 0.3[c,d]

[a] Mice were treated for 5 days with 3.0 μmol of DES. Controls received corn oil. Mice were inoculated intravenously with 10^4 *L. monocytogenes* cells 4 days after the final DES treatment.
[b] Each value represents the mean ± 1 standard error for at least eight mice.
[c] Significantly different from uninfected controls ($P < 0.05$) as determined by the LSD test based on a one-way ANOVA.
[d] Significantly different from infected controls ($P < 0.05$) as determined by the LSD test based on a one-way ANOVA.

TABLE 3. Response of ConA blast cells from DES-exposed mice to IL 2

Blast cell source[a]	[^{3}H]TdR incorporation (cpm × 10^3)[b] by cells treated with:		
	Medium (controls)	IL 2 (U/ml)	
		250	500
Control mice	0.4 ± 0.1	7.6 ± 0.8	20.8 ± 1.2
DES-treated mice	0.6 ± 0.1	5.9 ± 1.2	14.0 ± 4.8

[a] Mice were treated for 5 days with 3.0 μmol of DES. Controls received corn oil. Four days later, the spleens were removed, and blast cells were prepared as described in Materials and Methods.

[b] Each value represents the mean ± 1 standard error for four sets of blast cells prepared from the spleens of individual mice.

both DES and IL 2 were lower than in mice treated with DES alone.

Peritoneal macrophages from mice exposed to 3.0 μmol of DES were cultured in the presence of LPS to determine if estrogen exposure affected IL 2 production by depressing the production of IL 1. IL 1 production was quantitated in supernatant fluids by examining their ability to support the proliferative response of normal thymocytes to PHA. Macrophages from DES-treated mice were not suppressed in their ability to produce IL 1 and, in fact, were slightly more stimulatory to thymocyte proliferation than were control supernatants (Table 4).

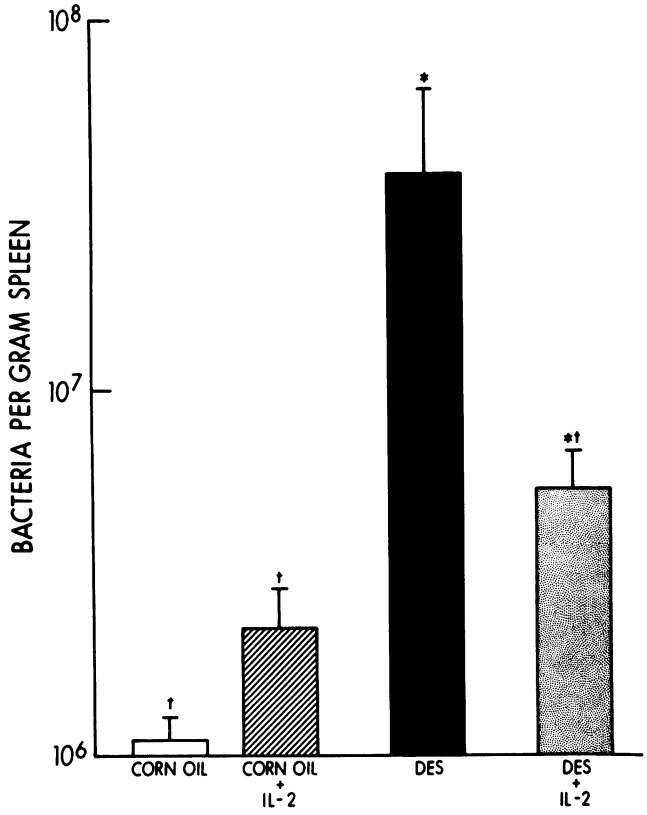

FIG. 2. Effect of IL 2 on susceptibility to *L. monocytogenes* after DES exposure. Mice were inoculated intravenously with 10^4 *L. monocytogenes* cells 4 days after treatment with 0.4 μmol of DES. On the day of infection, 1,500 U of IL 2 was injected intraperitoneally. Each value represents the mean ± 1 standard error for eight mice. Asterisks indicate significant differences from mice treated only with corn oil ($P < 0.05$) as determined by the LSD test based on a one-way ANOVA. Daggers indicate significant differences from mice treated only with DES ($P < 0.05$) as determined by the LSD test based on a one-way ANOVA.

DISCUSSION

The ability of mice to resist *L. monocytogenes* infection depends primarily on cell-mediated immune responses. Recovery is thought to require the accumulation of large numbers of macrophages at infective foci (28, 32) and macrophage activation by T-cell-produced lymphokines (15, 19). The immunological alterations associated with estrogen exposure are consistent with decreased resistance to *Listeria* strains resulting from altered macrophage and T-cell functions (reviewed in reference 25). Indirect evidence that estrogens disrupt the T-cell-mediated immune response to *L. monocytogenes* but not the bactericidal properties of nonimmune macrophages was provided in earlier studies in which the growth of bacteria was monitored in the spleens of estrogen-exposed mice (5, 34). Substances directly toxic to macrophages may result in greater numbers of bacteria in infective foci as early as 24 h after infection. Compounds toxic to components of the cell-mediated immune system are evidenced by greater numbers of bacteria in infective foci only later in the infection, when recovery requires T-cell activation (37). As shown previously (5, 34), the number of *L. monocytogenes* cells in the spleens of estrogen-treated mice is greater than the number observed in control mice on days 4 and 7 but not day 1 after infection. This indicates that estrogen-mediated alteration of the immune response to *L. monocytogenes* is mediated through the disruption of T-cell function and not of the bactericidal properties of resident macrophages. Furthermore, we have also shown that estrogen exposure does not decrease the bactericidal capacity of nonimmune macrophages (O. J. Pung and M. I. Luster, unpublished observations).

The effects of estrogens on T cells are multifold. For example, estrogens produce thymic involution characterized by depletion of cortical thymocytes (1, 6). Estrogens also affect T-cell function, as evidenced by suppressed delayed hypersensitivity and in vitro lymphoproliferative responses to allogeneic leukocytes and T-cell mitogens (22, 24). In vitro exposure to estradiol depresses the ability of splenic lym-

TABLE 4. Effect of DES exposure on the ability of macrophages to produce IL 1

Source of test supernatant[a]	[^{3}H]TdR incorporation (cpm × 10^3)[b] at indicated dilution of test supernatant	
	1:2	1:4
Medium	5.0 ± 0.4	ND[c]
Control cells	21.6 ± 3.0	13.9 ± 0.2
DES-treated cells	29.4 ± 3.0	25.0 ± 4.2[d]

[a] Mice were treated for 5 days with 3.0 μmol of DES. Three days later, supernatants containing IL 1 were prepared by culturing adherent peritoneal exudate cells from control or treated mice in complete medium containing 50 μg of LPS per ml. The medium contained LPS at amounts equivalent to those present in supernatants from cultured adherent cells.

[b] IL 1 production was quantitated by measuring the proliferative response of control thymocytes to test supernatants and 2 μg of PHA per ml. Each value represents the mean ± 1 standard error for two experiments with thymocytes pooled from 10 mice.

[c] ND, Not done.

[d] Significantly different from control cells ($P < 0.05$) as determined by the LSD test based on a one-way ANOVA.

phocytes to produce IL 2 (10). In the present studies it was noted that whereas splenic-lymphocyte numbers increased dramatically in control mice exposed to *L. monocytogenes*, splenic-lymphocyte numbers in infected DES-exposed mice failed to rise above the level observed in uninfected controls (Table 1 and Fig. 1). The increase in splenic lymphocytes in infected controls was, to a large part, due to an augmentation of the number of splenic T cells. Although macrophage accumulation at infective foci primarily results from an influx of circulating monocytes (32), the increase in splenic T cells during infection is thought to be mainly due to T-cell proliferation in the spleen (33, 39). The present studies demonstrated that estrogens inhibit the proliferation of splenic T cells during infection, an observation which probably correlates with the inability of splenic T cells from estrogen-treated mice to proliferate in response to T-cell mitogens. It was also found that estrogens, although not affecting the ability of blast cells to respond to IL 2, lead to the inhibition of IL 2 production by these cells (Tables 2 and 3). It is reasonable to suggest, therefore, that the depression of T-cell proliferation described above is a consequence of an estrogen-induced defect in IL 2 production.

Several lines of evidence support a role for IL 2 in recovery from *L. monocytogenes* infection. First, *L. monocytogenes*-sensitized Lyt 1^+23^- cells in the presence of IL 2 clonally expand to produce an enlarged population of cells capable of transferring *L. monocytogenes* immunity (18), and this population increases in the spleens of *L. monocytogenes*-infected mice (39). Second, Lyt 1^+23^- cells are potent producers of IL 2 (8, 16) and, in the presence of IL 2, proliferate to produce lymphokines, including macrophage-activating factor (3) and migration inhibitory factor (17), vital to recovery from *Listeria* infections.

Inoculation of mice with IL 2 partially reversed the effects of estrogen on resistance to *L. monocytogenes* infection. This suggests that the ability of estrogens to depress IL 2 production is directly related to the increase in the susceptibility of mice to the bacterium. Presumably, a decrease in the ability of lymphocytes from estrogen-exposed mice to produce IL 2 would depress the proliferation of *L. monocytogenes*-sensitized T cells and, as a result, depress the production of lymphokines responsible for effector-cell accumulation, macrophage activation, and granuloma formation at infective foci. This would permit bacterial growth to proceed relatively unchecked. Depression of the production of lymphokines such as migration inhibitory factor or chemotactic factor may be reflected by the previously demonstrated inability of estrogen-exposed mice to accumulate monocytes at infective foci at the same rate as control mice (34).

To investigate potential mechanisms by which estrogens might depress IL 2 production, we examined the effects of DES on IL 1. IL 1 plays an important role in the induction of IL 2 by serving as a second signal for T-cell activation (20). In the absence of IL 1, proliferation of antigen-sensitized T cells is inhibited and IL 2 production is depressed (20). It was found, however, that the ability of macrophages from DES-exposed mice to produce IL 1 was not impaired (Table 4). There are other mechanisms by which estrogen exposure might decrease IL 2 production; for example, there is evidence that DES decreases total splenic-T-cell numbers and, more specifically, depresses the percentage of Lyt 1^+23^- cells relative to Lyt 123^+ and Lyt 1^-23^+ cells (13). As Lyt 1^+23^- cells produce IL 2 (8), a decrease in their numbers, combined with an increase in the regulatory suppressor-T-cell population, would result in decreased IL 2 production. In light of the evidence that estrogens alter thymic hormones (9, 23, 24, 36), it should be noted that thymic hormones and IL 2 act in concert for normal maturation of intrathymic lymphocytes and that thymus humoral factor promotes IL 2 production (2, 38).

In conclusion, increased susceptibility to *L. monocytogenes* after exposure of mice to estrogens such as DES is not mediated by alterations in nonimmune macrophage functions. Rather, estrogens appear to inhibit both the in vitro proliferative response of spleen cells to T-cell mitogens and T-cell proliferation in the spleens of *L. monocytogenes*-infected mice. This is due to depressed IL 2 production, the importance of which is demonstrated by partial restoration of normal host resistance in estrogen-treated mice by IL 2.

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