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Alterations of the Antibody Response Following *In Utero* Exposure to Diethylstilbestrol

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Diethylstilbestrol (DES), a nonsteroidal estrogenic substance, is an environmental contaminant used both as a therapeutic agent in humans and an extratherapeutic agent in promoting growth of livestock. DES is also a recognized stimulant of the reticuloendothelial system (RES) and causes increased hepatic (KELLY et al., 1962; SLJIVIC et al., 1975) and increased (STEVEN and SNOOK, 1975) or decreased (SLJIVIC et al., 1975) splenic phagocytic activity of macrophages. The close association between the RES and the humoral immune response would suggest that DES would alter the antibody response. In this respect the effects of estrogens on the antibody response have been reported to vary from depression (BATCHELOR and CHAPMAN, 1965) and no effect (PEETOOM et al., 1970) to stimulation (BROOME and LAMMING, 1959). Several of these studies suggested that the alterations in the antibody response were due to increased or decreased splenic phagocytic activity (SLJIVIC et al., 1975; BATCHELOR and CHAPMAN, 1965), however, genetic (STERN and DAVIDSOHN, 1955) and humoral factors (NELSON et al., 1967) may play an influence. In a recent study estrogens were shown to directly interact with immunocompetent cells and stimulate mitosis resulting in an enhanced antibody response (KENNY and DIAMOND, 1976).

The present studies were undertaken to determine the effects of a single administration of DES in the prenatal period on the immune response to sheep red blood cells (SRBC) and E. coli 0127 lipopolysaccharide (LPS) which are thymic-dependent and thymic-independent antigens, respectively. For many agents the prenatal organism is more sensitive to chemical injury than the corresponding adult (MCLACHLAN and DIXON, 1976). In this respect, it was reported in human studies that among young females with clear-cell adenocarcinoma of the vagina, there was an increased use of DES during pregnancy compared with mothers of control subjects (HERBST et al., 1971). Recent studies have suggested that the mouse may be an appropriate model to study the transplacental effects of DES (MCLACHLAN et al., 1975).

MATERIALS AND METHODS

Date bred pregnant Swiss-Webster mice (Charles River) were injected with 0.1 mg of DES (Sigma) subcutaneously in corn oil on day 16 of gestation. Controls were injected with corn oil alone.

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Offspring were immunized intraperitoneally with either 0.2 ml of a 10% suspension of SRBC or 0.1 ml of a 0.1 mg/ml solution of E. coli 0127 LPS (Difco) at 7 weeks of age. For secondary LPS responses, mice were boosted with an additional 0.01 mg of LPS 3 weeks following the primary immunization. LPS immunized mice were sacrificed 4 days after immunization and SRBC immunized mice were sacrificed 5 days after immunization. Spleens were aseptically removed and sieved through a brass screen (U.S. Series No. 60) into sterile RPMI 1640. The spleen cell suspensions were counted on a Coulter Counter (Model ZB, Coulter Electronics, Inc., Hialeah, Fla.) and IgM or 19s plaque forming cells (PFCs) were enumerated according to the method of DRESSER and GREAVES (1973) as modified by VEIT and MICHAEL (1972). IgG or 7s PFC were developed by the addition of an optimal concentration of mouse anti-IgG antiserum. Statistical analysis was performed on the geometric means of transformed data employing the analysis of variance procedures (SNEDECOR and COCHRAN, 1967).

RESULTS

The number of SRBC and E. coli 0127 LPS PFC in sex segregated groups of control and in utero exposed mice is presented in Table 1. In utero exposure to DES did not alter the antibody response to SRBC at seven weeks of age. In contrast, LPS PFCs were decreased in DES treated females ($P<0.05$) and significantly increased in DES treated males ($P<0.01$) when compared to control groups. The enhanced action of DES on direct (IgM) LPS PFC in male mice could also be demonstrated during the secondary response but antibody suppression was not observed in females. The inability of mice to produce an IgG antibody response to LPS in these studies is consistent with other published data (SILVERSTEIN et al., 1965).

TABLE 1

Effect of in utero exposure to DES on the immune response to SRBC and E. Coli 0127 LPS

	Number Tested		PFC/10^6 Spleen Cells (± SE)			
			Treatment-Sex			
			0 Females	0 Males	0.1 mg Females	0.1 mg Males
1^o SRBC	32		431(±23.8)	448(±118.4)	413(±59.2)	365(±39.1)
1^o LPS	56		407(±43.3)	359(± 42.6)	294(±44.6)	986(±84.2)
2^o LPS	24	19s	256(±21.5)	256(± 10.0)	253(±23.8)	533(±49.5)
		7s	0	0	0	0

Any two treatment-sex groups not underscored by the same line are significantly different; $P<0.05$.

DISCUSSION

The mechanisms by which *in utero* exposure to low levels of DES enhanced the antibody response to LPS in male mice and suppressed the antibody response in females is unknown. In earlier studies by others, pretreatment of mice with high levels of DES shortly before immunization suppressed the antibody response to both SRBC (SLJIVIC and WARR, 1973) and type III pneumococcal polysaccharide (SLJIVIC and WARR, 1974), however, this suppression was related to decreased splenic phagocytic activity. In the present studies, it would appear unlikely that the alterations in the immune response are due to increased or decreased RES activity, since the antibody response to SRBC was normal while only the LPS response was affected by DES. It is well documented that LPS is one of the least macrophage dependent antigens and SRBC the most macrophage dependent (KUNIN, 1962). If the RES was responsible then the SRBC antibody response would have been more adversely affected than the LPS. Furthermore, DES was not detected in offspring, as young as 1 day old, from mothers treated with 0.1 mg of DES daily on days 10-16 of gestation (unpublished observations).

Although sex differences in the immune response to many antigens, including *E. coli* LPS, has been reported, these differences are usually reflected by enhanced immune responsiveness in females (KENNY and GRAY, 1971). That this phenomenon is related to estrogenic activity has been suggested in one study by the fact that the ability of young female mice to respond to low doses of *E. coli* LPS antigen increases with maturity and is lost following ovariectomy. Secondly, increased antibody responses to *E. coli* antigens occurs in young females when treated with low levels of estrogen 1 day prior and up to 3.5 days following antigen administration. Lastly, spleen cell cultures from *E. coli* antigen stimulated male mice have significantly increased antibody producing cells when incubated in the presence of estradiol (KENNY and DIAMOND, 1977). These studies further suggest that the increased antibody production associated with low levels of estrogens is not due to stimulation of the RES but rather through estrogens acting directly on immunocompetent cells, either by stimulating mitosis or accelerating differentiation of these lymphoid cells as is similar to the effects of DES on fetal cells (UCHIKAWA et al., 1970). Thus, it is possible that exposure to DES during certain periods of fetal development stimulates or selectively programs a particular population of lymphoid cells, particularly those involved with T-independent antigen responses, resulting in a relatively long term alteration in the antibody response.

The observation that males had enhanced antibody production to LPS while females had suppressed antibody synthesis was not

totally unexpected. It has been shown that while low levels of estrogens may stimulate antibody synthesis (KENNY et al., 1976), slightly higher levels are suppressive (SLJIVIC and WARR, 1974) and even higher concentrations are thymolytic (BATCHELOR and CHAPMAN, 1965). The sex differences in antibody response may reflect differences in the ability of males and females to bind DES to their estrogen receptors. DES is known to bind to estrogen cell receptors and females have quantitatively more of these receptors than do males (O'MALLEY and MEANS, 1973). This difference in total body estrogen receptors was found, at least in part, due to the large number of receptors in the female reproductive tract. Thus, the total amount of DES in circulation in the fetal male may be greater than that in the fetal female and affects programming of immune function differently in the two sexes.

Alternatively, alterations in circulating sex steroids in mice prenatally exposed to DES may play a persistent role in modifying immune response. The report of male sex hormone regulation of the Ss locus in the H-2 complex (PASSMORE and SHREFFLER, 1971) lends support to this hypothesis; the Ss protein has been recently demonstrated to be the fourth component of complement (MEO et al., 1975).

SUMMARY

The antibody response to SRBC and E. coli 0127 lipopolysaccharide were determined in offspring from mice exposed in utero to diethylstilbestrol. The antibody response to SRBC, a T-cell dependent antigen, was similar in control and exposed animals. In contrast, the LPS antibody response was suppressed in treated females and enhanced in treated males. These studies indicated that in utero exposure to DES alters the humoral immune system to T-independent antigens.

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