Table 4
Spleen weight and subpopulations of splenic lymphocytes in Experiment 2

Groups		Spleen (mg)	T cells (%)	Naive T cells (%)	Memory T cells (%)	B cells (%)	NK cells (%)
Young	♂-C	92.9±12.6	15.8±1.8	31.6±0.7	67.6±0.7	76.4±1.6	3.5±0.3
	♂-L1	98.8±8.2	15.7±1.7	37.8±3.9	61.3±3.8	74.1±1.3	3.0±0.1
	♂-L2	102.5±6.3	17.7±1.1	39.6±1.1*	59.6±1.1*	69.1±0.8*	2.9±0.1
Young	♀-C	83.1±3.6	21.3±2.4	43.2±2.9	56.0±2.8	59.3±3.3	4.8±0.3
	♀-L1	89.6±0.6	24.4±3.0	45.2±0.9	54.2±0.9	58.0±1.8	4.3±0.1
	♀-L2	88.6±7.3	27.2±1.1*	47.6±2.2	51.7±2.2	55.0±1.5	4.3±0.1
Old	♂-C	104.8±10.2	20.4±0.4	12.8±0.6	86.1±0.7	67.1±1.0	2.8±0.3
	♂-L1	108.0±14.8	23.6±1.8	14.3±2.1	85.0±2.1	58.8±4.3	2.1±0.3
	♂-L2	81.0±11.6	26.0±1.1*	12.2±1.2	87.1±1.1	59.0±0.4*	2.7±0.2
Old	♀-C	114.6±15.3	28.1±3.1	17.0±4.9	82.3±5.1	52.5±5.7	2.9±0.3
	♀-L1	122.1±15.3	18.8±1.1*	12.9±1.6	85.7±1.4	52.9±0.9	4.7±0.8*
	♀-L2	103.0±13.9	28.2±1.7	10.6±1.7	88.6±1.7	47.2±5.4	4.6±0.9*

C, L1 and L2, same as shown in Table 2. Asterisk (*) indicates statistically significant difference ($P < 0.05$) as compared with control. Naive and memory T-cells were percents in the CD4 positive T-cell population.

Immune functions were monitored in three parameters (Fig. 2). When comparing levels of anti-SRBC antibody response between L1 and L2, the levels were higher in L2 than in L1 in young male, but vice versus in young female. No great change was observed in old mice. Con A stimulation in young female mice showed a trend of increase in L2 than in L1. NK-activity increased much in L1 than in L2 in young females.

Expression of estrogen receptor in cells of immune system were checked (Fig. 3). Both ER-α and -β were strongly expressed in the ovary. ER-α was expressed also strongly in the thymus and slightly in spleen. Among thymocyte subpopulations, the most strongly expression of ER-α was observed in $CD4^+CD8^+$ double positive cells. Furthermore, T cells, B cells, macrophages and dendritic cells expressed ER-α

4. Discussion

In our first experiment, we employed pharmacological doses of DES (3.0–15.0 μg/kg). It confirmed the results of previous reports that high doses of DES suppressed immune functions in mice, such as severe thymic hypocellularity, a decrease in $CD4^+CD8^+$ cells with a concomitant increase in $CD4^-CD8^-$ cells as well as decrease in percentage and total number of T cells in the spleen (Smith and Holladay, 1997). H1 (3 mg/kg BW) dose of DES seems to be anabolic because of the body weight gain. Whereas H2 (15 mg/kg BW) dose seems to severely induce liver metabolic enzymes and stressful so that adrenals are hypertrophic and thymus becomes atrophic. Thymic atrophy was mainly due to decrease in $CD4^+CD8^+$ subpopulation whish is known to have highest sensitivity to corticosterone receptor-mediated apoptosis among CD4/8 subpopulations (Wiegers et al., 2001). Gonads can be hypertrophic through hypothalamus-pituitary-gonad feedback mechanism as a consequence of possible high clearance of steroid hormones by liver enzymes induced. The spleen weight gain may be a rebound hypertrophy after transient decrease by corticosteroid stimuli. The component that is responsible for the spleen weight gain could not be specified in this study. These confirmed features are in concordance with the reports such as DES suppresses cell-mediated immunity in vivo and in vitro (Luster et al., 1984; Pfeifer and Patterson, 1986), subchronic exposure to DES significantly increased the mortality of female mice after Listeria infection (Pung et al., 1984), and estradiol or DES suppresses the process of self-healing from infections induced by challenges of plasmodium chabaudi malaria in mice, but does not influence the survival rate of mice once acquired immunity to it (Benten et al., 1992).

In our second experiment, we looked into the DES effects in lower doses. In contrast to the high dose effect in the experiment 1, low dose of DES induced different responses by age (young and old) and by sex (male and female). Even though these changes were unexpectedly complicated, it is interesting to note there is an inverse relationship between two parameters. In other words, one parameter is higher in L1 (lower dose) than in L2 (higher dose) and the other is vice versus. One example would be the relation of thymus and adrenal gland. The fact that the inverse relation of thymus and adrenal were seen even in the condition that each showed non-monotonous dose-responses would indicate that the corticosteroid-mediated effects are superior to the age/sex-dependent regulations mechanisms. The inverse relation was also seen in immune functions (anti-SRBC, Con A), although the connecting mechanisms is not clear form our data. The possible explanation for the inverted relation in general would be that the homeostatic regulation mechanisms are holding but in different offset values that is a function of age and sex. On the other hand, the uniform changes among all age/sex groups, such as seen in $CD4^+CD8^+$ subpopulation may be the direct estrogenic effects by DES.

The endocrine disruptor issue can be recognized as a type of receptor mediated toxicity. The receptor mediated response is considered to be different from traditional toxicology that basically handles high dose findings and extrapolate the findings down to low dose ranges for risk assessment. This traditional approach totally depends upon an assumption that the dose response curves are monotonous at any dose range. However, the receptor biology in general is non-monotonous, due to either receptor down regulation, homeostatic feedback responses or cross talks between other signaling systems. Additionally, the signaling by receptor systems are often amplification systems, leading to an idea that the effective dose ranges are much lower than that of the traditional toxicology. In this respect, present DES study at low dose range provides us with an insight that receptor mediated toxicology are able to monitor in endocrine-immune interactive endpoints.

In conclusion, we confirmed and extended the influence of DES at pharmacological high dose and also showed the influence of physiological low dose upon the immune functions in age and sex dependent manner. This study indicate that low dose effect of exogenous estrogens to immune function should be assessed with caution as an integral of all age and sex, and in expectation of non-monotonous dose-response relationship.

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