Both treatments increased B-cell upregulated protein expression of GL7, CXCR5, and CXCR4. Splenic B cells and enhanced GC reactions, as shown by zone B cells were examined. Both chlordecone and E2 activated and E2 treatment on splenic germinal center (GC) and marginal pathways in mediating this effect. The effects of chlordecone and E2 indicate that chlordecone is not functioning simply as an estrogen mimic with respect to effects on the immune system. Similarities in the effects of chlordecone and E2 on specific immune functions, such as diminished apoptosis in GC B cells, may provide valuable clues regarding key events in the acceleration of autoimmune by E2, chlordecone, and other agents.

Key Words: autoimmunity; estrogen; chlordecone; spleen; splenocytes; systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) occurs predominantly in women, with the greatest risk of disease occurring during childbearing years (Lahita, 1996). A variety of factors—including genetic, environmental, and viral influences—are postulated to contribute to the development of SLE (D’Cruz, 2000), but the factors contributing specifically to increased risk in women are unclear. There is evidence to suggest that sex hormones have an important role in gender bias in SLE. This evidence includes studies in murine SLE models that show an effect of endogenous estrogen status on the rate of progression and severity of disease. For example, in female (NZB x NZW)F1 mice, ovariectomy delays development of elevated autoantibody titers and immune complex glomerulonephritis, while estradiol (E2) treatment restores the time course of autoimmunity to that seen in ovary-intact animals (Sobel et al., 2005). In (NZB x NZW)F1 males, the time to development of SLE is longer than for females but can be decreased by either castrog compounds or administration of estrogen (Roubinian et al., 1978). Estrogen treatment has also been demonstrated to increase the rate of onset of disease and mortality in lupus-prone MRL lpr/lpr mice (Carlsten et al., 1990, 1992).

Although far from conclusive, a number of clinical studies also suggest a role for sex hormones in SLE. The onset of SLE typically occurs after menarche and the disease subsides after menopause (Barrett et al., 1986; Sandborg, 2002). Increased SLE flares associated with pregnancy have been reported (Petri et al., 1991; Urowitz et al., 1993), perhaps due to protracted increases in estrogen. A recently completed clinical trial of combined estrogen and progesterone hormone replacement therapy (HRT) in menopausal SLE patients (the Safety of Estrogen in Lupus Erythematosus National Assessment) found that patients receiving HRT had significantly more lupus flares than patients given placebo (Buyon et al., 2005). The dose of estrogen and clinical status of the patient are important, as more recent study by the same group suggests that for stable, mild to moderate SLE, low doses of exogenous estrogen as given in oral contraceptives are reasonably safe (Petri et al., 2005).

The effects of estrogen on the immune system are complex, and a variety of mechanisms have been postulated through which estrogen might trigger or exacerbate autoimmunity. Examples include overexpression of autoantigens through estrogen-induced enhancement of transcriptional processes, hyperresponsivity to autoantigens by estrogen-enhanced Th2 cytokine
duction, and estrogen suppression of apoptosis of autoreactive T and B cells (for recent reviews, see Grimaldi et al., 2005; Lang, 2004; Sekigawa et al., 2004). Evidence suggests that estrogen effects in the spleen may be particularly important. Treatment of BALB/c mice transgenic for a rearranged IgG antibody specific for double-stranded DNA (R4A-IgG2b BALB/c) with implanted, sustained-release E2 pellets resulted in the appearance of IgG2b anti-DNA antibody titers. No increase in anti-DNA antibody titers was observed in mice treated with control pellets, indicating that autoreactive B cells exposed to estrogen in vivo were able to escape apoptosis and undergo activation (Bynoe et al., 2000). Subsequent experiments found that E2 treatment diminished B-cell receptor–mediated apoptosis in transitional B-cell populations (Grimaldi et al., 2002) and that most of the activated autoreactive B cells were from the splenic marginal zone (Grimaldi et al., 2001). Other studies have also suggested the importance of the marginal zone in producing autoreactive B cells leading to SLE in (NZB x NZW)F1 mice (Wither et al., 2000).

The germinal center (GC) of the spleen is another important site for deletion of autoreactive B cells. It appears that there is clonal deletion of GC autoreactive B cells (Han et al., 1995; Shokat and Goodnow, 1995) and that upregulation of Bcl-2 leads to increased autoreactive B-cell survival (Kuo et al., 1999). E2 treatment has been shown to increase Bcl-2 expression in GC cells (Bynoe et al., 2000), suggesting that this too may be an important site of action of estrogen in the spleen with respect to increasing autoreactive B cells.

We have recently shown that treatment of female (NZB x NZW)F1 mice with chlordecone, an organochlorine pesticide with estrogenic effects (Hodges et al., 2000; Okubo et al., 2004), accelerated the rate of development of SLE (Sobel et al., 2005). Because doses of chlordecone in humans resulting from environmental exposure have not been measured, it is difficult to determine how chlordecone doses producing more rapid onset of autoimmunity in mice compare with human exposures. However, comparison of chlordecone doses producing various adverse effects in mice indicate that autoimmune effects are among the most sensitive, i.e., they occur at doses of chlordecone at or below those required to produce other forms of toxicity. A similar but weaker effect on SLE was produced by E2 treatment at a dose rate of 0.028 mg/kg/day (Sobel et al., 2005), leading to speculation that chlordecone affects autoimmunity through its estrogenicity. However, doses of chlordecone capable of significantly decreasing time to onset of SLE produced little or no estrogenic response in the classical uterine hypertrophy assay. Consequently, it is unclear whether chlordecone is sufficiently potent as an estrogenic agent to be a functional estrogen mimic at doses relevant to autoimmunity. If it is not, this would suggest that chlordecone possesses a different mode of action with respect to its effect to accelerate development of SLE.

To address this question, a series of experiments were conducted in which the effects of chlordecone on the immune system were compared with E2 in ovariectomized (NZB x NZW)F1 mice. Morphological, phenotypic, and functional end points related to the spleen and splenic B cells were the focus of these experiments given the importance of the spleen as a target for estrogen effects leading to autoimmunity. The principal objective of the study was to determine whether chlordecone, at doses affecting autoimmunity, produced estrogen-like effects on the spleen and splenic B-cell populations.

MATERIALS AND METHODS

Mice. Female (NZB x NZW)F1 mice (6–8 weeks old) were purchased from The Jackson Laboratories (Bar Harbor, ME). Mice were housed in a climate-controlled, specific pathogen-free barrier facility. Mice were kept in polycarbonate cages on corn cob bedding with free access to food and water throughout the study. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida.

Test materials and treatments. Chlordecone from Crescent Chemical (Islandia, NY) was formulated into 60-day sustained-release pellets by Innovative Research of America (Sarasota, FL). Each chlordecone pellet contained either 1 or 5 mg chlordecone. Pellets containing 0.05 mg 17β estradiol (E2) or matrix only (as controls) from Innovative Research of America were used in the study for comparison purposes.

At the beginning of the experiments, each mouse was surgically ovariectomized and allowed to recover for 2 weeks. Then a pellet containing chlordecone, E2, or matrix only was implanted sc above the shoulders under light methoxyflurane anesthesia. Mice tolerated the ovarioectomy and pellet implantation procedures without complications. Mice were euthanized 5–6 weeks after pellet implantation, and tissues were harvested for study. This duration of exposure was chosen to enhance the ability to distinguish primary from secondary effects. That is, mice were euthanized before the development of overt autoimmune pathology so that events leading up to, rather than resulting from, clinical lupus could be evaluated.

RNA and cDNA preparation. Splenic B cells were enriched from spleenocytes of chlordecone-, E2- and control pellet–treated mice by negative selection with a mouse B cell isolation kit, (Mitenyi Biotec, Auburn, CA) according to the manufacturer’s directions. RNA from purified B cells was extracted by TRIZOL reagent (Invitrogen, Carlsbad, CA), and the concentration of RNA was measured by spectrophotometry. RNA (1 μg) was then treated with DNase I (Invitrogen) to remove genomic DNA and reverse transcribed to cDNA using Superscript II First-Strand Synthesis System (Invitrogen) for RT-PCR according to the manufacturer’s guidance.

Real-time PCR. Gene expression was determined by real-time PCR using SYBR green (Applied Biosystems, Foster City, CA). Primers were designed as follows: Bcl-2 forward AGTACCTGAACCGGCATCTG, reverse CAGGTATGACCCAGAGTGA; Shp-1 forward CGAAACGTGCATAGCAAGA, reverse GACGATGCCCAGATCACTT; β-actin forward CGGCC-TAGCTCTAGACAAAT, reverse GTCACCATTTCCTTGGCAGTT. Briefly, one microliter of cDNA from the preparation as described above was added to a reaction mixture containing 3mM MgCl2, 0.2mM deoxynucleoside triphosphate mixture, 0.025 U of AmpliTaq Gold, SYBR Green dye (Applied Biosystems) and a final volume of 25 μl. Amplification conditions were as follows: 95°C (10 min), followed by 45 cycles of 94°C (15 s), 60°C (25 s), 72°C (25 s), with a final extension at 72°C for 8 min. Transcripts were quantified using the comparative (2^−ΔΔCt) method.

Spleen morphology. Spleen tissue was fixed in neutral buffered 10% formalin for at least 24 h, trimmed, processed, embedded in paraffin, sectioned at 4–6 μm, and stained with hematoxylin and eosin. Spleen morphology was...
examined by light microscopy. The principal difference in morphology among treatment groups was in density and distribution of cells within the periarteriolar sheaths. Slides were examined in blinded fashion by a pathologist and scored using the following criteria: minimal changes; 0; mild to occasionally moderate; 1; moderate overall; 2; moderate and rarely extensive; 3; extensive overall with rare GCs; 4; and extensive overall with discrete, large GCs; 5.

**Flow cytometry.** Flow cytometry was performed with a CyAn ADP Analyzer (Dako Ft. Collins, CO). Single-cell suspensions from spleen were stained with mixtures of antibodies to the following surface markers: B220, IgM, CD19, CD21, CD24, CD44, CD69, CXCR4, CXCRI, ICAM-1, VCAM-1, MHC Class II, B7.2, and GL7. Intracellular staining was performed as described by Merino et al. (1994). Cells were incubated with rabbit anti-Bcl-2-PE or rabbit IgG-PE as isotype control. All antibodies were purchased from BD Biosciences Pharmingen (San Jose, California, USA).

Flow cytometry data analysis was accomplished with FCS Express V3 software (De Novo Software, Ontario, CA). In some cases, the expression of some molecules did not appear as two distinctive populations. In these cases, the median level of expression was normalized to the control mice, and the percentages of cells that showed a higher value compared with this median for different treatments were displayed.

**Aptosis assay.** B cells were enriched from splenocytes of chlordene- or control pellet–treated mice by negative selection with a mouse B-cell isolation kit (Miltenyi Biotec) according to the manufacturer’s directions. Purified B cells (1 × 10^6/ml) were grown in duplicate in complete RPMI 1640 medium, with or without 1 μg/ml lipopolysaccharide (LPS), at 37°C and 5% CO₂ for 24 h. Cells were stained for surface markers, and apoptosis was assessed by flow cytometry with the Annexin V-PE and 7-AAD apoptosis detection kit I (BD Biosciences Pharmingen) according to the manufacturer’s instructions. GC B-cell apoptosis was determined by Annexin V-PE staining on freshly prepared GL7 + B cells.

**Proliferation assay.** Purified splenic B cells at (2 × 10^5 per well) were cultured for 72 h in complete RPMI 1640 medium at 37°C and 5% CO₂, with or without added LPS stimulation (1 μg/ml). B-cell proliferation was assessed by testing [³H]TdR incorporation (ICN Biomedicals, Costa Mesa, CA) over the last 18 h of culture. Proliferation index was measured as the ratio of stimulated to unstimulated thymidine incorporation.

**Statistical analysis.** Statistical analyses were mainly conducted using the software GraphPad Prism, version 4.00 (GraphPad, San Diego, CA). Comparisons of spleen morphology scores were made using the nonparametric Kruskal-Wallis test. For flow cytometric results that were normalized to the control group data, the control group data were excluded in the ANOVA as the control group mean was outside the Gabriel comparison intervals. Treatment means were identified using Gabriel’s comparison intervals method (Gabriel, 1978). If the control group mean was outside the Gabriel comparison intervals, the control group data were excluded in the ANOVA as the treatment means were identified using Gabriel’s comparison intervals method.

### RESULTS

Spleen morphology was examined after 6 weeks of treatment with either E2 or chlordene. No significant lesions were found in red pulp from mice treated with E2 sustained-release pellets (0.05 mg per 60-day release pellet; approximate dose of 0.024 mg/kg/day), chlordene (5 mg per 60-day release pellet; approximate dose 2.4 mg/kg/day), or matrix-only (control) pellets (Figs. 1A–C). The density of mixed cells in splenic cords was moderate, including minimal red cells. Extramedullary hematopoiesis ranged from minimal to mild. Some differences in white pulp were observed among the treatment groups. Control mice (Fig. 1A) showed less dense cuffs of periarteriolar lymphocytes and no GCs compared with mice treated with either chlordene or E2 (Figs. 1B and 1C). Diffuse periarteriolar lymphatic sheaths were moderate to extensive in mice treated with either E2 or chlordene but minimal in the white pulp of control mice. Scoring of the distribution of the periarteriolar lymphatic sheaths from 0 to 5 (minimal to extensive; see “Materials and Methods” section for details) showed the increases in chlordene and E2-treated mice to be statistically significant (Fig. 1D). In general, control mice had minimally or mildly populated lymphatic nodules devoid of GCs. Chlordene- and E2-treated mice had moderately to extensively populated lymphatic nodules. Some chlordene-treated mice had no GCs or discrete marginal zones while others had rare GCs with discrete marginal zones. Mice treated with E2 were more likely to have GCs with discrete marginal zones.

To study the effects of chlordene on B lymphocytes, seven-color flow cytometry was performed on splenocytes. Chlordene exposure increased the level of activation markers CD44 and CD69, which was similar to observations in the E2-treated group (Fig. 2A). MHC Class II and the costimulation marker B7.2 were also increased by both treatments (Fig. 2A). In the case of MHC Class II expression, treatment groups were normalized as the ratio of the geometric mean of the treated mouse to that of the control mouse from that day’s group. E2, but not chlordene treatment, significantly increased the CD138⁻B220⁺ mature splenic plasma cell population (Fig. 2B), while neither treatment affected CD138⁺B220⁺ immature plasma cells (data not shown).

Since the GCs play an important role in the maturation of humoral immune response resulting in the production of high-affinity, class-switched B cells, including autoreactive B cells, we looked more closely at GC B cells. There was a dose-dependent increase in the percent of CD19⁺ splenic B cells expressing GL7, a marker of GC B cells, in response to chlordene (Fig. 3A). Along with the increase in GL7⁺ B cells, there was also a corresponding increase in expression of CXCR4⁺ and CXCR5⁺ B cells (Fig. 3B). These two chemokine receptors are important in the organization of GCs and the tracking of cells within them (Allen et al., 2004). When gated on GL7⁺ GC B cells, CXCR4 and CXCR5 expression levels were also increased in the chlordene-treated group (data not shown). Enhanced GC reactions were also seen in the E2-treated group (Figs. 3A and 3B).

In order to characterize further the behavior of B lymphocytes and the GC B-cell subset after chlordene exposure, isolated B cells were tested for proliferation and apoptosis under different conditions. The expanded GC B-cell population could have derived from decreased basal apoptosis. Under both “stimulated” and “unstimulated” conditions, isolated splenic B cells from both chlordene- and E2-treated mice after 24 h
of culture underwent apoptosis at reduced rates compared to control-treated mice (Fig. 4A). This decreased rate of apoptosis was also seen in freshly stained GL7⁺ GC B cells, both in the chlordecone and E2 groups (Fig. 4B). In contrast, there were no consistent changes in B-cell proliferation as measured by the ratio of stimulated to unstimulated thymidine incorporation (Fig. 4C).

The expressions of Bcl-2, ICAM-1, and VCAM-1 are known to be related to the apoptosis of GC B cells (Bynoe et al., 2000; Grimaldi et al., 2002; Koopman et al., 1994), and Shp-1 overexpression can favor the survival of autoreactive B cells (Grimaldi et al., 2002). Protein expression of Bcl-2, ICAM-1, and VCAM-1 in B cells was measured by flow cytometry, and gene expression of bcl-2 and shp-1 was tested by quantitative real-time PCR. E2 treatment has been shown to upregulate Bcl-2 expression on splenic B cells of R4A-IgG2b BALB/c transgenic mice, but this has not been reported for nontransgenic mice. E2 increased expression of Bcl-2 in splenic B cells of ovariectomized (NZB x NZW)F₁ mice, a feature shared by chlordecone exposure (Fig. 5A). Chlordecone treatment increased both ICAM-1 and VCAM-1 expression level on total CD19⁺ B cells as well as GC B cells (Fig. 5B), both were similar to the effects of E2 treatment. At the gene level, chlordecone treatment significantly enhanced bcl-2 (Fig. 6A) and shp-1 (Fig. 6B) expression, an effect that did not reach statistical significance in the E2 treatment group.

As it has been reported that E2 exposure can expand the marginal zone B-cell population in R4A-IgG2b BALB/c transgenic mice (Grimaldi et al., 2001), we examined the impact of sustained exposure to chlordecone or E2 in vivo on the distribution of splenic B-cell subsets in young, ovariectomized (NZB x NZW)F₁ mice. Flow cytometric analysis revealed...
FIG. 2. Activation of splenic B cells by chlordecone and E2. Splenocytes from chlordecone-, E2-, and placebo-treated mice were analyzed by flow cytometry using antibodies that recognize CD19, B220, CD69, CD44, B7.2, MHC Class II, and CD138. B cells were identified as the CD19$^+$B220$^+$ population. The expression of all the molecules except MHC Class II was based on the percentage of the positive population, as described in "Materials and Methods" section. The expression of MHC Class II was based on the median fluorescence intensity and was normalized to the control group. (A) Chlordecone and E2 effects on CD69, CD44, B7.2, and MHC Class II expression. (B) Chlordecone and E2 effects on the percentage of plasma cell population. Mature and immature plasma cells were defined as B220$^+$CD138$^-$ and B220$^+$CD138$^+$, respectively. Any treatment that caused significant increase compared with the control group was marked with a star symbol. For experiments where data were normalized to control values, Treatment groups with no statistically significant differences were marked with the same letter (either a or b), while groups with significant difference were marked with different letters.
a significant decrease in percentage of B220\(^+\)/CD24\(^+\) immature B cells and an increased percentage of B220\(^+\)/CD24\(^-\) mature B cells in E2-treated mice (Fig. 7A), which was similar to the results seen in transgenic mice. The reduction of immature B cells by E2 was due to a decrease in the percentage of CD21\(^+\)/CD24\(^+\) transitional type 1 (T1) B cells, which represent the most immature splenic B-cell population and give rise to transitional type 2 (T2) B cells. The CD21\(^+\)/CD24\(^+\) transitional T2 population, which differentiates into the mature splenic B-cell populations, was only slightly reduced in E2-treated mice. Chlordecone treatment had no appreciable influence on the distribution of these populations. The

**FIG. 3.** Chlordecone and E2 effects on splenic GCs. (A) Representative dot plots of GC marker GL7 expression on B cells in chlordecone-, E2-, and placebo-treated mice, and the overall result of GL7 expression in different groups. (B) Chemokine receptors CXCR4 and CXCR5 expression in B cells in different treatments.
increasing percentage of mature B cells seen in the spleens of mice treated with E2 was derived from a significant increase in CD21⁺CD24⁻/CD24⁺ marginal zone B cells as there was no statistically significant increase in the percentage of CD21⁻/CD24⁻/CD24⁺ follicular B cells (Fig. 7B). In contrast, chlordecone treatment had no effect on the percentage of marginal zone or follicular B cells.

**DISCUSSION**

There is increasing evidence that environmental factors play important roles in the regulation of the immune system (Hess, 2002; van Loveren et al., 2001). We have previously shown that organochlorine pesticides can accelerate autoimmunity in both ovariectomized and ovary-intact female (NZB x NZW)F₁ mice, with changes in the time course and magnitude of disease similar to that seen with treatment with the sex hormone E2 (Sobel et al., 2005, 2006). In the present studies, we investigated whether the chlordecone and E2 likely affected the same pathways, basing our studies on known effects of E2 on the immune system. Both chlordecone and E2 activated splenic B cells and in particular, enhanced GC B-cell reactions as shown by upregulated protein expression of GL7, CXCR5, and CXCR4. Although GC cells account for only a small percentage of the total spleen cell population, it is an important area where negative selection for autoreactive B cells occurs (Pulendran et al., 1995; Shokat and Goodnow, 1995).

The fate of B cells in the GC is associated with some important molecules. Chemokine receptors CXCR4 and CXCR5 are important for the organization of the GC. It has been reported that CXCR4 is critical in localizing centroblasts to the dark zone, while CXCR5 helps direct cells to the light zone (Allen et al., 2004). Enhanced CXCR4 and CXCR5 expression...
Analysis of chlordecone and E2 regulation of Bcl-2, ICAM-1, and VCAM-1 protein expression in B cells. Levels of these molecules in B cells were determined by flow cytometry. As these molecules are expressed at some level by virtually all B cells, analysis was conducted by median expression levels, as described in “Materials and Methods” section. (A) Representative histogram of Bcl-2 expression on B cells from chlordecone- (heavy line), E2-, (heavy line) and control pellet-treated (light line) mice, and the overall result of Bcl-2 expression in different groups. (B) ICAM-1 and VCAM-1 expression in total B cells and GL7+ GC B cells. See the legend to Figure 2 for an explanation of the symbols.
on GC B cells by chlordecone and E2 treatment might facilitate the trafficking of autoreactive cells to the GC. Previous reports showed that the adhesion molecules ICAM-1 and VCAM-1 can mediate the attachment of GC B cells to the follicular dendritic cells via the integrins LFA-1 and VLA-4. This association of B cells and follicular dendritic cells is thought to be important for driving affinity maturation (Hedman et al., 1992; Koopman et al., 1994). The enhanced expression of cell adhesion molecules ICAM-1 and VCAM by chlordecone and E2 treatments might transfer a stronger signal from follicular dendritic cells to the B cells, which postpones the apoptosis of autoreactive B cells. It is also possible that E2 or chlordecone have direct effects on B-cell survival. In fact, reduced B-cell apoptosis was seen in both unstimulated conditions and following LPS stimulation. Moreover, reduced GC B-cell apoptosis was also observed in freshly isolated B cells. However, when B cells were stimulated with anti-IgM, no significant change was observed (data not shown). One possible explanation for these observations is that the resistance to apoptosis is not a feature of B-cell receptor-mediated signaling. However, this seems unlikely, as it has been previously shown that E2 decreased B cell receptor-mediated apoptosis as measured by caspase-3 expression (Grimaldi et al., 2002). A more likely possibility is that these culture conditions resulted in a high degree of variability that prevented statistical significance from being achieved.

We also found Bcl-2 expression was increased in B cells. Enhanced Bcl-2 expression is believed to be responsible for reduced apoptosis and increased survival of autoreactive cells (Kuo et al., 1999). Although both treatments increased Bcl-2 protein levels as measured by flow cytometry, E2 treatment showed the stronger effect. However, chlordecone treatment (5 mg pellet) caused a larger increase in bcl-2 mRNA levels, as measured by real-time PCR, suggesting that E2 and chlordecone may have differing effects post-transcriptionally. Shp-1, a protein tyrosine kinase, is an important molecule in the inhibitory receptor signaling pathway (Plas and Thomas, 1998). E2 has been reported to increase Shp-1 in the BALB/c transgenic mouse model, and elevated Shp-1 has been speculated to increase the threshold of B-cell signaling and impair mechanisms of tolerance in autoreactive B cells (Grimaldi et al., 2002). In agreement with the reported results on transgenic BALB/c mice, exogenous E2 exposure increased shp-1 B-cell gene expression in ovariectomized but otherwise unmanipulated (NZB x NZW)F1 mice, a feature shared with chlordecone. Taken together, our results suggest that chlordecone and E2 might help autoreactive B cells escape negative selection by influencing one or more checkpoints of the GC reaction.

Important differences between the effects of chlordecone and E2 were also seen. Exposure to chlordecone did not significantly increase the percent of mature splenic plasma cells, which is in contrast to E2 treatment. Plasma cells in (NZB x NZW)F1 mice are known to accumulate abnormally in the spleen and to have decreased migration to the bone marrow (Erickson et al., 2003). While it is possible that chlordecone altered the balance of plasma cells in the spleen and bone marrow, the chlordecone-treated group secreted less autoantibodies compared with the E2 group after 6 weeks of treatment (data not shown), arguing against this possibility. However, our previous results showed similar IgG immune complex deposition in the kidney (Sobel et al., 2005). It is possible that chlordecone has altered the fine specificities of autoantibody production, a possibility that cannot easily be explored with the present model.

E2 treatment has also been reported to enhance marginal zone B-cell populations significantly in a transgenic BALB/c mouse model, and the autoreactive B cells displayed a marginal

**FIG. 6.** Analysis of chlordecone and E2 regulation of Bcl-2 and Shp-1 gene expression in isolated B cells. B cells were enriched from splenocytes of chlordecone-, E2-, and control pellet–treated mice by negative selection. bcl-2 (A) and shp-1 (B) gene expression was determined by using quantitative real-time PCR.
FIG. 7. Analysis of chlordecone and E2 effects on B-cell subsets. Splenic B-cell subsets were identified by using B220, CD19, CD21, and CD24 as markers. (A) E2, but not chlordecone treatment, reduced immature CD24⁺ population and increased mature CD24⁻ population. (B) The increased mature B cells in E2-treated group was due to a significant increase in CD21⁺CD24⁻ marginal zone B cells but not the CD21⁺CD24⁺ follicular B-cell population, while the decrease in immature B-cell population came from a decrease in CD21⁻CD24⁺ transitional type 1 B cells but not CD21⁻CD24⁺ transitional type 2 B-cell population. These effects were not shared by chlordecone treatment.
zone phenotype (Grimaldi et al., 2001). We verified the finding of enhanced marginal zone B-cell population by E2 treatment in the (NZB × NZW)F1 mouse model. In contrast, chlordecone did not change the percentage of this population, which might suggest chlordecone effects on autoimmunity are not through its effects on marginal zone B cells. However, it is still possible that chlordecone changed the repertoire of marginal zone B cells by increasing the relative number of B cells with autoreactive specificities without affecting the overall percentage. Again, a transgenic “knock-in” model would be needed to address this possibility.

In summary, the results of this study indicate that chlordecone and E2 share common effects on splenic morphology and GC B-cell populations, including reduced rates of B-cell apoptosis. This suggests that impaired deletion of autoreactive B cells in the spleen may be an important contributor to the effects of both compounds to accelerate development of autoimmunity in the (NZB × NZW)F1 mouse. The effects of chlordecone and E2 on the spleen were not identical, however. Differing effects were seen on marginal B-cell populations. These differences indicate that chlordecone is unlikely to be functioning simply as an estrogen mimic with respect to the immune system and possesses activity independent of its estrogenic properties. On one hand, complexity in immune effects of chlordecone will make it challenging to determine the mechanisms responsible for its acceleration of autoimmunity. On the other hand, identification of similarities and differences between chlordecone and E2 effects on various immune targets may provide the opportunity to obtain valuable clues as to the key events leading to endocrine and chemical-induced exacerbation of SLE. While this study focused on chlordecone and E2 effects on various immune targets may provide the opportunity to obtain valuable clues as to the key events leading to endocrine and chemical-induced exacerbation of SLE. While this study focused on chlordecone and E2 effects on various immune targets may provide the opportunity to obtain valuable clues as to the key events leading to endocrine and chemical-induced exacerbation of SLE.

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SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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