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PAPER

Differential effects of estrogen and prolactin on autoimmune disease in the NZB/NZW F1 mouse model of systemic lupus erythematosus

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Estrogen and prolactin have been shown to modulate autoimmunity in the NZB/NZW F1 (B/W) mouse model of systemic lupus erythematosus (SLE). However, estrogen stimulates prolactin secretion. The goal of this study was to examine differential effects of estrogen and prolactin in the female B/W mouse model of SLE. B/W females were manipulated to create combinations of low and high concentrations of serum estrogen and prolactin. Hyperprolactinemic mice with either low or high serum estrogen levels had accelerated development of albuminuria at 24 and 32 weeks of age compared to normal and hypoprolatinemic mice. High estrogen/high prolactin mice also had a higher percentage of anti-DNA antibodies compared to mice in the low estrogen/low prolactin and the high estrogen/low prolactin groups. IgG levels were not significantly different between groups. Mean survival was shortest in the high estrogen/high prolactin group (34 ± 1.0 weeks) and longest in the high estrogen/low prolactin group (42 ± 1.2 weeks; $P < 0.05$). High levels of serum estrogen were associated with depressed *in vitro* lymphoproliferation and IL-2 production. This study suggests that high prolactin levels in either high or low serum estrogen states are associated with accelerated autoimmunity in the B/W mouse. This study further demonstrates that high estrogen levels do not accelerate murine SLE when the prolactin-stimulating property of estrogen is suppressed by bromocriptine. Further investigation of hormonal interactions in autoimmunity will provide a better understanding of hormonal immunoregulation and, perhaps, lead to improved clinical application of hormonal immunomodulation.

Keywords: lupus; estrogen; prolactin; hormone; mice

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown cause characterized by T cell cytokine abnormalities, B cell hyperactivity, antinuclear antibodies, and immune complex formation/deposition, leading to arthritis, skin rash, serositis, and glomerulonephritis. The strongest risk factor for the development of SLE is female sex. The disorder is most common in young women, reaching a peak female:male ratio of 9:1 during the reproductive years.[1–4] SLE exacerbations have been associated with menstruation,[6] administration of oral contraceptives,[7] pregnancy,[8,9] altered estrogen and androgen meta-bolism,[10–12] and hyperprolactinemia.[13,14]

The NZB × NZW F1 (NZB/NZW) mouse develops autoimmune disease analogous to human disease, and female mice develop autoimmune disease earlier than male mice. T cell abnormalities and B cell hyperactivity in this murine model lead to hypergammaglobulinemia, anti-DNA antibodies, and immune complex formation/deposition. The animals die with glomerulonephritis and vasculitis.[15] Estrogen, progesterone,[16,17] and prolactin[18] accelerate autoimmune disease in this model. These observations support the concept that female sex hormones stimulate SLE disease activity. However, *in vivo* hormonal modulations of the immune system and autoimmunity are confounded by the interdependent

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actions of the pituitary-gonadal hormone axis. Estrogen stimulates prolactin secretion[19–22] and is responsible for differences in prolactin concentrations between women and men. Conversely, high levels of prolactin suppress estrogen production.[23] These interactions confound the interpretations and conclusions regarding specific immunoregulatory effects of hormones. It is not clear whether estrogen is directly immunostimulatory, because estrogen has the potential to stimulate prolactin release. The increased levels of circulating prolactin may be immunostimulatory. This study was designed to examine differential contributions of estrogen and prolactin to autoimmune disease activity in the female B/W mouse model of SLE.

Materials and methods

Animals

NZB/NZW mice were bred and maintained in the Research and Education Building of the G.V. (Sonny) Montgomery VA Medical Center in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care. Mice were housed in plastic cages on hardwood bedding, given water and rodent chow *ad libitum*, and exposed to controlled lighting (12 h light and 12 h dark).

Manipulation of estrogen and prolactin levels Hormonal modulation of individual NZB/NZW mice was achieved by ovariectomy, injections of 17-β estradiol, pituitary transplantation, and/or injections of bromocriptine (BRC). Surgical ovariectomies were performed on all mice in the study at 6 weeks of age.

Estrogen Low-dose estrogen treatment, designed to produce physiologic levels of estrogen in control mice, was given as injections of 17-β estradiol (0.32 µg s.c. every 4 d) in 0.1 ml sesame oil vehicle (Sigma Chemical, St Louis, MO). High physiologic serum estrogen concentrations were acheived by s.c. injections of 17-β estradiol (3.2 µg every 4 d) in 0.1 ml sesame oil.[24–27] Serum concentrations of estradiol and progesterone were measured using the Estradiol Enzyme Immunoassay Kit and the Progesterone Enzyme Immunoassay Kit (Caymen Chemical, Ann Arbor, MI).

Prolactin Serum prolactin concentrations were increased in designated groups by transplanting syngeneic pituitary glands, which is a reliable and reproducible means of creating chronic hyperprolactinemia.[28] Pituitary glands were removed aseptically from female NZB/NZW donors and two glands were implanted under the renal capsule of each recipient as described previously.[18] The other animals received a sham operation in which a sliver of syngeneic liver was implanted under the renal capsule. Designated groups of mice were treated with daily s.c. injections of BRC (10 mg/kg). The purpose of this treatment was to suppress serum prolactin without altering the concentrations of other hormones.[29] Prolactin concentrations were measured by radioimmunoassay (RIA), using reagents obtained from the National Institutes of Health pituitary hormone program (Dr Parlow) as previously described.[18]

Longevity study

Study protocol and monitoring Six-week-old female NZB/NZW mice were assigned at random to five groups, surgical procedures were performed and injections were begun and continued throughout the lifespan of each mouse. Groups of mice that were manipulated to produce either low or high estrogen concentrations and either low or high prolactin concentrations are shown in Table 1. Mice were examined daily for signs of disease. Mice were

Table 1 Hormonal manipulations of longevity study

Group	*No. of subjects (n)*	*Estrogen (µg/mouse)*	*BRC (10 mg/kg)*	*Pit transplant*	*Estrogen (pg/ml)*[a]	*Prolactin (ng/ml)*[a,b]	*Progesterone (pg/ml)*[a]
LE/LP	12	oil	yes	sham	34 ± 25	16 ± 1 (4 ± 2)	1926 ± 828
LE/HP	14	oil	no	yes	65 ± 30	185 ± 28 (68 ± 18)	1246 ± 203
NLE/NLP	10	0.32	no	sham	68 ± 28	215 ± 23 ND	1191 ± 250
HE/LP	10	3.2	yes	sham	335 ± 96	162 ± 44 (13 ± 7)	851 ± 98
HE/HP	12	3.2	no	yes	273 ± 119	97 ± 24 (206 ± 8)	495 ± 102

[a]Sera were collected by orbital bleeding under anesthetic conditions at 8 weeks of age.
[b]Prolactin values in parentheses are from sera of trunk blood collected from unanesthetized decapitated mice at 13 weeks of age.
LE/LP = low estrogen/low prolactin; LE/HP = low estrogen/high prolactin; NLE/NLP = control mice with hormonal manipulations designed to produced normal estrogen and normal prolactin; HE/LP = high estrogen/low prolactin; HE/HP = high estrogen/high prolactin.

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weighed, anesthetized, and bled from the orbital venous plexus at 8, 16, 24, 32, and 40 weeks of age. The capillary tubes of blood were centrifuged, the parts of the tubes that contained serum were cut free with a triangular file, and the parts of the tubes that contained serum were plugged on both ends and stored at $-80°C$. Moribund animals were bled, sacrificed, and autopsied.

Tests for renal disease At each bleeding interval and at necropsy, urine was expressed from the bladder and tested for albuminuria with Albustix® (Ames Co, Elkhart, IN). Albuminuria was ranked 0, Trace, 1+ (30 mg/dl), 2+ (100 mg/dl), 3+ (300 mg/dl), or 4+ ($\geq$ 200 mg/dl). Blood urea nitrogen (BUN) was assessed using an end-point colorimetric reaction using the BUN (Endpoint) 50 kit (Sigma Chemical, St Louis, MO) and BUN standards per manufacturer's instructions.

Anti-DNA antibodies and IgG concentrations Anti-DNA antibodies were tested in serum samples diluted 1:100 using the ELISA method described by Zhou *et al*.[30] Absorbance was measured at 405nm and optical density (OD) values >0.1 were considered positive. Serum IgG concentrations were analyzed by ELISA.[18] Absorbance was measured at 405 nm and total serum IgG levels were quantitated by plotting absorbance against a standard curve.

Histopathology Complete necropsies were performed as previously described[18] and included gross and microscopic examination of thymus, lymph nodes, spleen, salivary glands, lungs, heart, liver, bladder, and ovaries to determine the cause of death.

Interval study

DNP-KLH immunization In a separate short-term study, 6-week-old B/W females were divided into five groups of 6–8 mice each, oophorectomized, and treated as described in the longevity study. At 12 weeks of age mice were immunized with the hapten-carrier protein, dinitrophenyl keyhole limpet hemocyanin (DNP/KLH) (Calbiochem-Novabiochem Corporation, La Jolla, CA). Ten days post-immunization, mice were decapitated without anesthesia, blood was collected, and serum anti-DNP IgG antibodies were analyzed by ELISA.[31] The same samples of serum were used for quantitation of prolactin concentrations in unanesthetized mice.

Cell culture Spleens were removed aseptically and minced gently to a single cell suspension in complete RPMI medium without phenol red (Cellgro TM, Mediatech/Herdon, VA) supplemented with 5% fetal calf serum (Sigma Chemical, St Louis, MO) and 1 mM L-glutamine (Cellgro™–Mediatech, Herdon, VA).

For assays of cytokine production, triplicate aliquots of 1×10^6 cells were dispensed into 24-well treated tissue culture plates (Costar, Cambridge, MA) and stimulated with 100 µg/ml keyhole limpet hemocyanin (KLH, Calbiochem-Novabiochem) or 1 µg/ml concanavalin A (ConA) for 24 h. The supernates were assayed for interleukin (IL)-2, IL-4, IL-6, and IFN-gamma using ELISA (Endogen Cytokine MiniKits™, Endogen, Cambridge, MA). Cytokine concentrations were quantitated by plotting sample absorbance against the standard curve.

For assessment of lymphocyte stimulation, triplicate aliquots of 1×10^5 cells were dispensed into 96-well treated tissue culture plates (Costar) and cultured with KLH in concentrations of 10, 100, or 1000 µg/ml, or with Con A 1 µg/ml in 5% CO_2 at 37°C for 72 h. In the final 4 h of incubation, cell proliferation was assessed using the CellTiter 96™ Aqueous Non-Radioactive Cell Proliferation Assay (Promega Corporation, Madison, WI), and absorbance was read at 450 nm.

Statistical Analyses Analysis of quantitative data was performed by ANOVA. The two-sided Fischer's exact test was used to compare two independent proportions. Survival data between groups was analyzed by the log rank test. Life spans of mice dying from injection accidents or anesthesia for serial bleedings were handled as censored data.

Results

Hormone concentrations

Serum estrogen, prolactin and progesterone concentrations were measured in mice bled under anesthesia in the longevity study. Table 1 illustrates estrogen levels at 8 weeks of age. Groups are designated as low estrogen/low prolactin (LE/LP), low estrogen/high prolactin (LE/HP), physiologic estrogen/physiologic prolactin (NLE/NLP, control), high estrogen/low prolactin (HE/LP), and high estrogen/high prolactin (HE/HP). Mean serum estrogen concentrations in the two low estrogen groups were 34 and 65 pg/ml. Mice that received 'physiologic' estrogen injections that

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were designed to induce physiologic levels of estrogen had a mean serum concentration of 68 pg/ml. The high estrogen groups had mean serum estrogen concentrations that were 335 and 274 pg/ml.

Serum progesterone concentrations were highest (mean values 1926 and 1246 pg/ml) in the LE/LP and LE/HP groups. The lowest mean progesterone values (851 and 495 pg/ml, respectively) were found in the HE/LP and HE/HP groups, which had the highest serum estrogen levels (Table 1).

Serum prolactin levels in the hormone manipulated groups are also shown in Table 1. Mean serum prolactin concentrations in the low prolactin groups were 16 and 162 ng/ml. The high prolactin groups had mean serum prolactin concentrations of 97 and 185 ng/ml. Serum obtained by bleeding the HE/LP group under anesthesia contained prolactin at a mean concentration of 162 ng/ml. We propose that this high value of prolactin was spurious. The stress of anesthesia stimulates prolactin secretion and elevates serum prolactin concentrations in rodents.[32] We have show previously that BRC effectively suppresses serum prolactin concentrations in unanesthesized NZB/NZW mice.[18] In the current study, we verified BRC suppression of prolactin by decapitating unanesthesized mice in the interval study groups and collecting trunk blood. In these serum samples, the mean prolactin concentration was 13 ng/ml in the HE/LP group, compared to 206 in the unanesthesized HE/HP group.

Renal disease

Figure 1 illustrates cumulative albuminuria in the longevity study mice. In the hyperprolactinemic groups (LE/HP and HE/HP), there was a trend to premature development of albuminuria ($>2+$) at 24 weeks of age compared to the control group and the two hypoprolactinemic groups. Accelerated albuminuria persisted in both groups of hyperprolactinemic mice at 32 weeks of age. The proportion of mice with albuminuria in the LE/HP and HE/HP groups at 32 weeks of age was significantly greater compared to the HE/LP group ($P < 0.05$ for both comparisons). In contrast, HE/LP mice had relatively low occurrence of albuminuria at 24, 32, and 40 weeks of age.

Mean BUN values did not differ significantly between groups throughout the study at the test points. All moribund mice had high serum levels (>30 mg/dl) of BUN, and this finding confirmed that they died with renal insufficiency (data not shown).

Anti-DNA antibodies and IgG

The percentages of mice in each group with anti-DNA antibodies (>0.1 OD at 405 nm; 1:100 sera dilution) are shown in Figure 2. At 16 weeks of age, occurrence of anti-DNA antibodies was relatively low in the LE/LP and HE/LP groups compared to the control group and the hyperprolactinemia groups. Proportions of mice with anti-DNA antibodies in the LE/LP group and the HE/LP group differed significantly from the proportion of mice with anti-DNA antibodies in the HE/HP group ($P < 0.05$ for both comparisons). At 24 weeks of age, percentages of mice with anti-DNA antibodies had increased in all groups and 100% of the HE/HP mice had anti-DNA antibodies. Eight weeks later, more than 80% of mice in each group had anti-DNA antibodies. Terminal sera from mice in every group had anti-DNA antibodies.

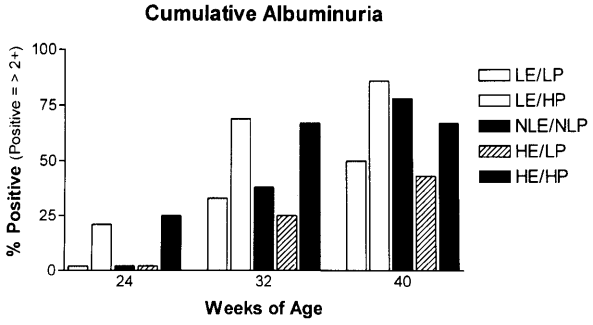

Figure 1 Cumulative albuminuria: Values at each time point are the percentage of total number of female NZB/NZW mice in each group that had $>2+$ albuminuria. LE/LP = low estrogen/low prolactin; LE/HP = low estrogen/high prolactin; NLE/NLP = control mice with hormonal manipulations designed to produced normal estrogen and normal prolactin; HE/LP = high estrogen/low prolactin; HE/HP = high estrogen/high prolactin. In the HE/LP group, there was a significantly lower percentage of mice with albuminuria at 32 weeks of age compared to LE/HP or HE/HP groups ($P < 0.05$ for both comparison).

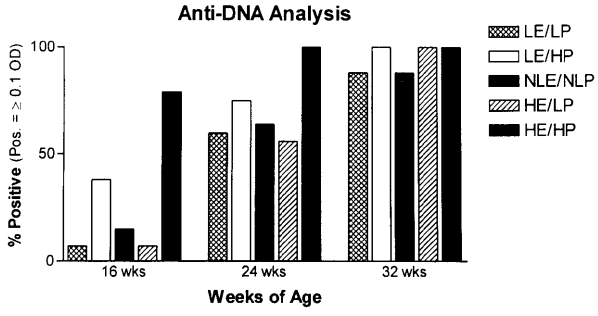

Figure 2 Percentage of mice with anti-DNA antibodies. Values at each time point are the percentage of female NZB/NZW mice in each group found to be positive for anti-DNA antibodies (OD 405 nm > 0.100). At 16 weeks of age LE/LP and HE/LP mice had significantly lower percentages of animals with anti-DNA antibodies compared to the HE/HP group ($P < 0.05$ for both comparison).

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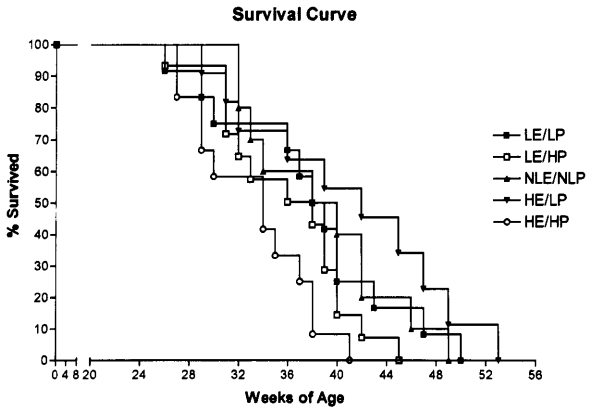

Figure 3 Survival curves for each group of hormonally manipulated female NZB/NZW mice. Accidental deaths (injection or bleeding) were handled as censored data. Survival curves between the HE/HP and HE/LP groups differed significantly ($P < 0.05$).

Mean serum IgG concentrations were not significantly different between hormonally manipulated groups at 16, 24 or 32 weeks of age (data not shown).

Survival

Figure 3 illustrates survival in the five groups of hormonally manipulated female NZB/NZW mice. Mean survival was longest (40 weeks) in the HE/LP group; this value did not differ significantly from the LE/LP group (38 weeks), the LE/HP group (36 weeks), or the control group (39 weeks). However, female NZB/NZW mice with high estrogen and high prolactin (HE/HP) had accelerated mortality with mean survival 33 weeks. Comparison of survival curves showed that survival in HE/HP mice was significantly shorter compared to the HE/LP group ($P < 0.05$). Differences between the groups were most pronounced when the animals were 42 weeks old. At that point, survival in the LE/HP and HE/HP groups was 15% and 0%, respectively, compared to 54% survival in the HE/LP group.

Anti-DNP antibodies

Serum anti-DNP antibodies did not differ signficantly between groups of hormone-manipulated mice.

Lymphocyte stimulation

In vitro lymphoproliferative response to KLH was not affected significantly by the *in vivo* manipulations

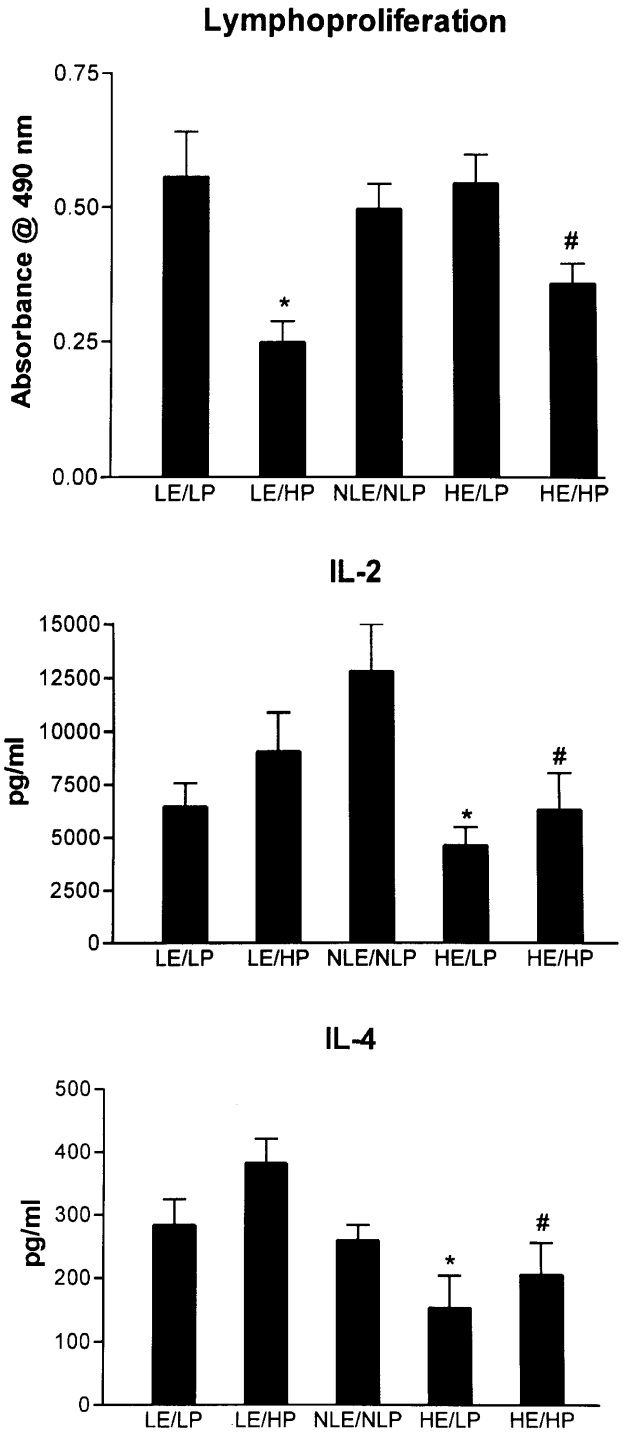

Figure 4 Lymphoproliferation: this graph illustrates the lymphoproliferative response to Con A in hormonally manipulated female NZB/NZW mice ($n = 6$–8/group) in the interval study. Results are expressed as mean ± s.e.m. of OD 405 nm as determined by MTT assay. LE/HP was significantly less than LE/LP, NLE/NLP, or HE/LP ($P < 0.001$ for each comparison). HE/HP was significantly less than LE/LP and HE/LP ($P < 0.05$ for each comparison). IL-2 concentrations (pg/ml) in supernatants after 24 h of stimulation with Con A ($n = 6$–8/group). HE/LP and HE/HP were significantly less than NLE/NLP (*$P < 0.01$; #$P < 0.05$). IL-4 concentrations (pg/ml) in Con A stimulated supernatants at 24 h. HE/LP and HE/HP were significantly less than LE/HP (*$P < 0.01$; #$P < 0.05$).

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(data not shown). In contrast, hormonal manipulation did modify lymphoproliferative responses to Con A. Figure 4A shows that hyperprolactinemic groups with either low estrogen or high estrogen levels demonstrated significant suppression of mean absorbance compared to control mice (LE/HP = 0.249 ± 0.039; HE/HP = 0.358 ± 0.038). When mean OD in the LE/HP group and mean OD in the HE/HP group were compared to the mean OD in the control group (0.498 ± 0.047), $P < 0.05$. In contrast, absorbance for the LE/LP group (0.558 ± 0.084) and the HE/LP group (0.547 ± 0.054) did not differ significantly from control mice (0.498 ± 0.047).

Production of cytokines

In vitro IL-2 and IL-4 production in response to Con A was modulated significantly by hormonal manipulation (Figure 4). The most dramatic response to hormonal manipulation was suppression of IL-2 production in Con A stimulated splenocytes from the mice with high estrogen, as shown in Figure 4. High estrogen groups had a suppression of IL-2 production compared to normal estrogen/normal prolactin mice (Figure 4B). IL-4 production (Figure 4C) was also suppressed significantly in both high estrogen groups compared to the LE/HP mice. *In vitro* IL-6 and IFN-gamma production in response to Con A and production of IL-2, IL-4, IL-6, and IFN-gamma in response to KLH did not differ between the groups (data not shown).

Discussion

Hormonal modulation of immunity and autoimmunity is complex and occurs at different levels. It is generally accepted that females have stronger immune response and are more prone to develop autoimmunity than males because hormones such as estrogen and prolactin are immunostimulatory. Estrogen has been shown to stimulate IFN-gamma production[33] and enhance B cell differentiation;[34,35] however, estrogen may also inhibit B cell differentiation.[36] Prolactin modulates IFN-gamma production[37,38] and B cell function[18,39], and progesterone increases production of Th2 cytokines.[40] Stimulatory and inhibitory feedback loops confound analyses of hormonal immuno-regulation *in vivo*, and individual hormones may have multiple, overlapping effects on different components of the immune system. Estrogen clearly stimulates pituitary secretion of prolactin.[19–21] Recently, it was shown that administration of high-dose estrogen resulted in marked elevation of serum prolactin concentrations and prolactinomas in NZB/NZW mice. Mortality from treatment with superphysiologic doses of estrogen is due to toxic effects on the genitourinary tract.[41] We manipulated serum estrogen and prolactin concentrations surgically and pharmacologically. These changes allowed us to begin to delineate effects of modest elevations of one hormone in the presence of low levels of the other hormone, and to compare these results with either low or high levels of both hormones.

All mice were ovariectomized to remove the major endogenous source of estrogen and progesterone. Subsequently, mice were injected with either vehicle or 17-β-estradiol every 4 d; two doses were used, 0.32 or 3.2 μg. These regimens were designed to simulate the murine estrous cycle, and to produce either low physiologic or high physiologic estrogen levels.[24–27] The serum estrogen concentrations that we measured (Table 1) verified that the desired estrogen concentrations were achieved. High estrogen groups were expected to have increased serum prolactin concentrations.[19,20] Therefore, BRC was administered to mice in the HE/LP group to suppress estrogen-induced release of prolactin. BRC was also administered to the LE/LP group to provide a comparable group with low serum levels of estrogen and prolactin. These manipulations resulted in the expected serum prolactin concentrations that were found in unanesthetized mice (Table 1).

Serum progesterone concentrations were related inversely to serum estrogen levels and had an extragonadal source (for example, the adrenal glands), as all mice were ovariectomized.

The hormonal manipulations had significant effects on autoimmune disease development and mortality in the female NZB/NZW mouse. The most dramatic effects were seen in the HE/HP NZB/NZW mice, which had accelerated albuminuria, anti-DNA antibody formation, and mortality (Figures 1, 2 and 3). Intermediate severity of autoimmune disease was observed in the LE/LP, LE/HP and control NZB/NZW mice. In contrast, the HE/LP group had delayed onset of autoimmunity and this finding suggested that elevated levels of estrogen were immunostimulatory only in a setting in which there was stimulation of prolactin release from the pituitary. Alternatively, BRC may have had immunosuppressive effects independent of its ability to inhibit pituitary release of prolactin.[42,43] It is known that dopamine and similar agonists may actually activate the estrogen receptor and its responsive genes.[44] Moreover, administration of exogenous prolactin reverses BRC-induced immunosuppression.[45,46] These facts imply that BRC has multifactorial immunosuppressive

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effects, but the major effects are related to its ability to suppress prolactin secretion. Our results confirm the concept that high serum prolactin concentrations, irrespective of high or low serum estrogen concentrations, stimulated autoimmune disease.

Low estrogen levels disrupt pituitary-gonadal axis feedback loops and lead to increased production of follicle stimulating hormone (FSH) and luteinizing hormone (LH).[47] It has been suggested that FSH and LH stimulate autoimmunity in female NZB/NZW mice.[48] Immune stimulation related to FSH and LH may explain the observation that ovariectomy alone does not ameliorate SLE in female NZB/NZW mice.[16,17] It is possible that increased pituitary secretion of FSH and LH increased the serum progesterone concentrations in the low estrogen groups in the current study.

The results of this investigation suggest that previously observed *in vivo* immunostimulatory effects of estrogen administration may be mediated through prolactin. Moreover, elevated serum estrogen alone did not appear to be immunostimulatory and may have suppressed autoimmune disease if its prolactin-stimulating properties were suppressed. The hormonal manipulations appeared to have effects on T cell function as assessed by lymphoproliferation and cytokine production. Direct effects on T:B cell collaboration were not detected by changes in serum anti-DNP antibody formation (data not shown). However, there were global defects in lymphoproliferation and cytokine production. Lymphoproliferation in response to Con A was suppressed in the hyperprolactinemic groups regardless of estrogen status compared to the other hormonally manipulated groups. Decreased responses to Con A were not paralleled by suppression of Con A-induced IL-2 production, as production of IL-2 was suppressed in the high estrogen groups (Figure 4). In contrast, Con A stimulated IL-4 production in spleen cells from the LE/HP mice and IL-4 production was lowest in the high estrogen mice, regardless of prolactin status. This effect was parallel to serum progesterone concentrations and was consistent with previously reported effects of progesterone[40] on IL-4. These data suggest that relative levels of female sex hormones alter *in vitro* cytokine production in response to mitogens. The dissociation between IL-2 production and lymphoproliferation in this model does imply that the hormones of interest have multiple effects on immunoregulation. There is little evidence for a direct suppressive effect of prolactin on IL-2 production,[49] and we propose that estrogen is primarily responsible for the defects in *ex vivo* NZB/NZW splenocyte lymphoproliferation and IL-2 production that have been reported by other investigators.[15,27] Mechanisms of hyperprolactinemic suppression of lymphoproliferation are currently unknown and remain to be investigated.

The findings in this animal study are consistent with recent clinical observations. Four women with hyperprolactinemia and suppressed serum estrogen concentrations and SLE have been described.[13] In addition, BRC-induced suppression of serum prolactin concentrations in patients with active SLE significantly reduced SLE disease activity, irregardless of estrogen status.[50]

In conclusion, this study confirmed previous reports that prolactin stimulates autoimmune disease activity in the female NZB/NZW mouse. Moreover, high serum estrogen concentrations were not immunostimulatory to murine SLE in the setting of BRC-induced suppression of circulating prolactin. While the findings of this study will require verification, they suggest that the concept of *in vivo* estrogenic immunoregulation must include both direct actions on immunocompetent cells as well as indirect actions mediated through prolactin. Further delineation of *in vivo* hormonal immunoregulatory interactions and mechanisms is crucial to our understanding of the sexual dichotomy of autoimmune diseases and to the practical and novel clinical applications of hormonal immunomodulation.

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