Prolactin modulates the naive B cell repertoire

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Prolactin is a peptide hormone produced by the anterior pituitary gland that is critical in lactation. Prolactin can also be produced by lymphocytes, and both B and T cells express prolactin receptors. These findings have suggested that prolactin has immunomodulatory functions. Studies in spontaneously autoimmune hosts have demonstrated a role for prolactin in augmenting autoreactivity. We chose to analyze prolactin effects on anti-DNA B cells in nonspontaneously autoimmune female BALB/c mice transgenic for the heavy chain of an anti-DNA antibody. Treatment with prolactin for 4 weeks induced a lupus-like phenotype with an increased number of transgene-expressing B cells, elevated serum anti-DNA antibody titers, and glomerular immunoglobulin deposits. Prolactin caused a decrease in the population of transitional B cells and an increase in mature follicular and marginal zone B cells. The DNA-reactive B cells had a follicular cell phenotype. Anti-DNA hybridomas demonstrated that prolactin alters selection of the naive B cell repertoire. The expansion and activation of anti-DNA B cells in prolactin-treated R4A-γ2b BALB/c mice was dependent on the presence of CD4+ T cells. Finally, treatment with prolactin was unable to break tolerance in R4A-γ2b transgenic C57Bl/6 mice, suggesting that responsiveness of the immune system to prolactin is genetically determined.


Introduction
Systemic lupus erythematosus (SLE) is a chronic inflammatory disease characterized by the production of autoantibodies directed toward a variety of nuclear antigens. The incidence of SLE is much higher in women than men, with a female to male ratio of 10:1 (1). This preponderance of SLE in women is thought to be mediated, in part, through the female sex hormones, estrogen and prolactin. The effects of these hormones at the cellular and molecular level are not well delineated. It is known that estrogen has immunostimulatory properties and that it stimulates prolactin secretion (2). It has also been shown that estrogen can exacerbate disease in spontaneous murine models of SLE only when prolactin is also present (3). We have recently demonstrated that the effect of estrogen on autoreactive B cells in a nonspontaneously autoimmune mouse model can be partially blocked by bromocriptine, an inhibitor of prolactin secretion (4). Studies in male and female NZB/W F1 lupus-prone mice demonstrated that hyperprolactinemia induced by transplantation of pituitary glands leads to disease exacerbation and early mortality (5, 6), whereas bromocriptine treatment improves survival (7).

Prolactin is a peptide hormone produced by the anterior pituitary gland that affects mammary growth and development. Over the past decade, it has been determined that prolactin can be produced at extrapituitary sites, including by lymphocytes (8, 9). It remains unclear whether the amount of prolactin secreted by lymphocytes is sufficient to affect the prolactin level in the serum. However, in a patient with acute myeloid leukemia, the leukemic cells secreted sufficient prolactin to cause hyperprolactinemia (10). Receptors for prolactin have been found on monocytes and B and T lymphocytes (11–17). Thus, prolactin can function in a paracrine and autocrine as well as an endocrine fashion.

The effects of prolactin on T cells have been studied more extensively than its effects on B cells. Prolactin has been shown to enhance the release of thymocytes from lymphoepithelial complexes in the thymus (18), to act as growth factors for T cells (19), to cause preferential development of CD4+ T cells (20), and to promote a Th1 response (21). Mouse helper T cell clones require prolactin as a cofactor for IL-2–driven lymphoproliferation (22), and anti-prolactin antibodies can inhibit T lymphocyte responses to mitogens (23).

Elevated prolactin levels have been found in 15–25% of patients with SLE (24–30). Furthermore, an association has been reported between hyperprolactinemia and anti-DNA reactivity in women less than 50 years of age (30). Some hyperprolactinemic
patients have prolactinomas (31) or secondary caus-
es of hyperprolactinemia such as hypothyroidism,
chronic renal failure, or medication-induced hyper-
prolactinemia (32, 33), but in most patients with
SLE, the cause of the increased prolactin levels can-
not be found.

Although attempts to correlate prolactin levels with
either global lupus activity (34–37) or specific organ
involvement (38–40) have yielded varying results, it
has been demonstrated that both nonstimulated and
mitogen-stimulated lymphocytes from patients with
lupus secrete more prolactin than control lympho-
cytes (41, 42). A single nucleotide polymorphism in
the upstream promoter of prolactin affects prolactin
transcription in lymphocytes; this polymorphism has
been shown to associate with SLE in a small cohort of
patients (43). It has also been speculated that alter-
ations in cyclic 2 amino 2 methyl propanol (AMP)
response-element binding family proteins might be
involved in the prolactin upregulation in the lympho-
cytes of patients with lupus (22). Small clinical tri-
als of bromocriptine, a drug that blocks prolactin
secretion by the anterior pituitary, have suggested a
beneficial effect in patients with SLE who have mild
and moderate disease activity (44, 45).

Our laboratory has been studying BALB/c mice
transgenic for the γ2b heavy chain of the R4A
anti–double-stranded (ds) DNA antibody (46). In
these mice, the R4A-γ2b heavy chain can associate
with the endogenous light chain repertoire, creating
a variety of transgene-encoded antibodies; some of
these bind DNA, whereas others do not. Three dis-
tinct populations of anti-dsDNA B cells have been
identified (47–50). The first is an ignorant or indif-
ferent resting B cell population that is not regulated
and produces nonpathogenic, low-affinity anti-
dsDNA antibodies (47). The second is an anergic pop-
ulation that produces high-affinity anti-dsDNA anti-
bodies that acquire a high affinity for DNA by somatic mutation (48, 49). The third is a deleted pop-
ulation of naive B cells in which unmutated germine
immunoglobulin genes encode high-affinity anti-
dsDNA antibodies. These deleted B cells are easily
detectable in R4A-γ2b/bcl-2 double-transgenic mice
and in R4A-γ2b NZB/W F1 mice (50).

In this study, we demonstrate that increased pro-
lactin breaks B cell tolerance in R4A-γ2b BALB/c
mice. Prolactin caused a lupus-like phenotype, char-
acterized by an expansion of the transgene-express-
ing B cell population, a significant elevation in serum
titers of anti-DNA antibody, and IgG deposits in the
glomeruli. The impact of prolactin on autoreactive B
cells was abrogated in the absence of CD4+ T cells,
demonstrating that the survival, expansion, and acti-
vation of anti-DNA B cells is T cell dependent. Final-
ly, we demonstrate that the immunostimulatory
effects of prolactin are genetically determined, as
demonstrated by the lack of response to prolactin in
R4A-γ2b C57Bl/6 mice.

Methods

Transgenic mice. R4A-γ2b BALB/c and C57Bl/6 mice
and CD4-deleted (knockout) R4A-γ2b transgenic
BALB/c mice were bred at the animal facility of the
Albert Einstein College of Medicine. The R4A-γ2b
BALB/c mice have been bred to the BALB/c back-
ground for well over 20 generations. R4A-γ2b
C57Bl/6 mice were generated by backcrossing the
R4A-γ2b transgene onto the C57Bl/6 background
for more than 15 generations. CD4-deficient mice
have been bred onto a BALB/c background for 10
generations before interbreeding with R4Aγ2b
BALB/c mice. Ovariectomized female mice (8–16
weeks of age) were used in these studies.

Prolactin treatment. The R4A-γ2b mice were injected
subcutaneously with 25 µg of recombinant mouse
prolactin (National Hormone and Peptide Program,
NIH, supplied by A. F. Parlow), 100 µg of ovine pro-
lactin (Sigma-Aldrich, St. Louis, Missouri, USA), or
100 µl of saline as a placebo each day for 4 weeks. Pro-
lactin levels in placebo- and prolactin-treated mice
were determined by RIA by the National Hormone
and Peptide Program at Harbor-UCLA Medical center
(Torrance, California, USA).

ELISAs. Immulon-2 plates (Dynex Technologies,
Chantilly, Virginia, USA) were coated with calf thymus
dsDNA as described. Culture supernatants normalized
to 5 µg/ml IgG2b or serum at various dilutions were
assayed for anti-dsDNA antibodies.

Flow cytometry. Splenocytes were isolated from pro-
lactin- and placebo-treated mice. After red blood cell
lysis, cells were stained with FITC-, phycoerythrin-, or
allophycocyanin-conjugated antibodies specific for CD19, γ2b, CD22, VCAM-1, SHP-1, Bcl-2, CD40, B 7.1 and B 7.2, CD21, CD23, and CD24 (HSA;
PharMingen, San Diego, California, USA) at 4°C for 30
minutes; the cells were then washed, fixed with 2%
paraformaldehyde, and analyzed by flow cytometry
(Becton Dickinson, Mountain View, California, USA).
For intracellular staining of Bcl-2 and SHP-1, cells were
permeabilized with 0.3% saponin.

ELISpot assay. Splenocytes pooled from prolactin- and
placebo-treated mice were labeled with anti-CD21
FITC and anti-CD23 phycoerythrin. Marginal zone
(CD21<sub>high</sub>/CD23<sub>low</sub>) and follicular
(CD21<sub>intermediate</sub>/CD23<sub>low</sub>) B cells isolated with a FACSVantage cell sorter
(Becton Dickinson) were added in serial dilution to
DNA-coated plates and incubated for 6 hours at 37°C.
Biotin-conjugated goat anti-mouse γ2b (Southern
Biotechnology, Birmingham, Alabama, USA) diluted
1:500 was added, followed by alkaline phosphatase–
conjugated streptavidin (Southern Biotechnology) at a
1:1000 dilution. The plates were developed with
5-bromo-4-chloro-3 indolyl phosphate substrate
(Sigma-Aldrich). DNA-reactive spots were counted
under a dissecting microscope.

Immunohistochemistry. Kidney sections were pre-
pared as previously described (51). Formalin-fixed,
paraffin-embedded kidney sections were stained with
biotinylated anti-mouse IgG antibody and developed with the alkaline phosphatase ABC detection kit (Vector Laboratories, Burlington, California, USA). Five-micrometer-thick frozen spleen sections from prolactin- or placebo-treated R4A-γ2b BALB/c mice were fixed in acetone for 5 minutes, blocked with 3% BSA/PBS for 30 minutes, and incubated for 30 minutes with a 1:200 dilution of 7-amino-4 methylcoumarin-3-acetic acid–labeled anti-IgM (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, USA) and Texas red–labeled anti-γ2b (PharMingen).

**B cell hybridomas.** Splenocytes were isolated from R4A-γ2b BALB/c mice after 4 weeks of treatment with ovine prolactin. Red blood cells were lysed, and isolated splenocytes were fused with NSO cells at a 2:1 ratio as previously described (52). Cells from positive wells were cloned, and their DNA reactivity was determined by DNA ELISA.

The anti-dsDNA antibody–producing hybridomas were screened for transgene expression by RNA dot blot using a 32P-labeled 210-bp DNA fragment that detects all members of the S107 V_H family (47). The S107 V_H–positive hybridomas were screened for expression of a V kappa light chain by RNA dot blot using a 230-bp probe for genes of the V kappa 1 family (48). V kappa light chains of anti-dsDNA–secreting hybridomas were sequenced. RNA was extracted, and RT-PCR was performed using a V kappa 1 FR1 primer and a kappa constant region primer as described (48). The PCR products were purified with the use of the Qiaquick purification kit (Qiagen Inc., Santa Clara, California, USA). V gene sequences were determined using an ABI 377 automated sequencer (Perkin Elmer, Applied Biosystems Division, Foster City, California, USA). Sequence

**Figure 1**

(a) Serum titers of anti-dsDNA antibodies. Fourteen R4A-γ2b BALB/c mice were injected subcutaneously with 100 µg of murine prolactin daily and 12 were injected with placebo daily for 4 weeks. Sera collected before initiation of treatment and weekly thereafter showed an increase in anti-DNA titer in placebo-treated mice. Anti-DNA reactivity assayed from serum obtained at the end of the treatment showed that prolactin-treated mice had significantly higher titers of anti-DNA antibody than placebo-treated mice (P = 0.023). OD 405, optical density at 405 nanometers. (b) Immunohistochemistry of kidney sections. Fourteen prolactin-treated and 12 placebo-treated R4A-γ2b BALB/c mice were sacrificed after 4 weeks of treatment. The kidneys were stained for IgG deposition. Anti-DNA deposits were found in prolactin-treated mice but not in placebo-treated mice. Representative sections from mice treated with prolactin or placebo are shown. Magnification, ×40.

**Figure 2**

Analysis of peripheral B cell lymphocytes. Splenocytes were isolated from seven murine prolactin–treated and seven placebo-treated mice after 4 weeks of treatment. (a) A higher percentage of B cells express γ2b in prolactin-treated than in placebo-treated mice (P = 0.01). The total number of γ2b cells in the spleens of prolactin-treated mice was 3.98 ± 0.84 x 10⁶ as compared with 2.3 ± 0.53 x 10⁶ in placebo-treated mice. (b) γ2b staining of splenocytes Representative dot plot showing an increased number of γ2b-expressing cells in prolactin-treated mice. (c) Localization of IgG2b-expressing B cells. B cell regions in the spleen were identified with anti-IgM staining (blue); γ2b-expressing B cells were labeled with anti-γ2b (yellow). The spleens of prolactin-treated mice displayed an expansion of the γ2b B cells, which were found mainly in the follicles. In spleens of placebo-treated mice, transgene-bearing B cells were localized to the T/B cell interface.
analysis was performed with Genetics Comparison Group Software (Madison, Wisconsin, USA).

Statistical Analysis. Standard statistical tests (mean value, standard deviation, two-tailed Student’s t test) were performed for data analysis.

Results

Anti-dsDNA antibody production in prolactin- and placebo-treated mice. We have previously demonstrated that estrogen can rescue a high-affinity DNA-reactive B cell population that is normally deleted in the R4A-γ2b BALB/c mouse (53). We have also demonstrated that increased anti-dsDNA titers in estrogen-treated mice can be abrogated by bromocriptine, thereby demonstrating that some of the immunostimulatory effects of estrogen require that prolactin be present (3). In order to determine whether prolactin itself can abrogate B cell tolerance, we analyzed anti-dsDNA serum titers in R4A-γ2b mice treated with murine prolactin. Fourteen female R4A-γ2b BALB/c mice were treated daily with prolactin, and 12 were treated with saline. Serum samples were collected on a weekly basis starting a day before initiation of treatment and analyzed for anti-dsDNA reactivity by ELISA. Prolactin levels were 68.3 ± 20.75 nanograms/ml (ng/ml) in prolactin-treated mice and 30.3 ± 19.7 ng/ml in placebo-treated mice, demonstrating that prolactin administration caused a twofold increase in serum concentration. By the fourth week of treatment, the titers of IgG2b anti-dsDNA antibodies increased significantly in prolactin-treated R4A-γ2b BALB/c mice (Figure 1a). There was no detectable IgM anti-DNA reactivity or IgG 1 anti-DNA reactivity.

Glomerular IgG deposition was detected in the kidneys of 11 of 14 prolactin-treated R4A-γ2b mice, suggesting that increased levels of prolactin led to secretion of potentially nephritogenic, high-affinity anti-dsDNA antibodies. Only 2 of 12 placebo-treated mice had any glomerular IgG, and these glomeruli were minimally affected (Figure 1b). These findings are consistent with the previous observation that unmanipulated R4A-γ2b mice effectively regulate the production of anti-dsDNA antibodies (46). The increase in anti-dsDNA antibody titer and the associated glomerular immunoglobulin deposition observed

Figure 3

B cell maturation. (a) Splenocytes from nontransgenic BALB/c mice treated with murine prolactin (n = 5) or placebo (n = 3) were stained for CD19, CD21, CD23, and HSA and were analyzed for T1, T2, marginal zone, and follicular subsets. B cell subsets were analyzed on the basis of data obtained with CD21 and HSA staining for the T1, T2, and follicular subsets and CD21 and CD23 staining for the marginal zone subset. (b) In prolactin-treated mice, the numbers of immature HSAint/CD21 +/CD23 + B cells were reduced (P = 0.006). In the mature HSAint B cell population, the numbers of follicular (CD21int/CD23int) B cells were increased (P = 0.006). (c) The marginal zone B cell subset (CD21int/CD21int) was increased in prolactin-treated mice (P = 0.005) (d) ELISpot assay of follicular and marginal zone B cells pooled from four ovine prolactin-treated or four placebo-treated mice demonstrated an increase in the number of spontaneously secreting DNA-reactive follicular B cells. MZ, marginal zone; Fo, follicula.

Figure 4

Anti-DNA B cells in CD4 knockout R4A-γ2b transgenic BALB/c mice. Splenocytes were isolated from seven murine prolactin–treated and seven placebo-treated CD4 knockout R4A-γ2b BALB/c mice after 4 weeks of treatment and were evaluated for γ2b expression and the number of spontaneously activated DNA-reactive B cells. (a) Prolactin- and placebo-treated mice displayed the same difference in the percentage of B cells expressing γ2b. The total numbers of γ2b cells in the spleens of prolactin- and placebo-treated mice were 1.82 ± 0.76 × 10^6 and 1.67 ± 0.51 × 10^6, respectively. (b) ELISpot assay showed that prolactin did not induce an increase in the number of anti-DNA antibody–secreting B cells (cells).
In prolactin-treated R4A-γ2b BALB/c mice demonstrate that prolactin can break tolerance sufficiently to produce a disease-related phenotype.

Peripheral B cell analysis. In the R4A-γ2b mouse, essentially all γ2b B cells express the transgene (48, 49). An expansion of the transgene-expressing B cell population was observed in R4A-γ2b BALB/c mice after prolactin treatment (Figure 2, a and b). Increased survival of transgene-expressing B cells was also confirmed by immunohistochemistry of splenic sections, with the transgene-expressing B cells localized mainly to the follicles (Figure 2c).

To investigate effects of prolactin on B cell development, we analyzed B cell subsets in prolactin-treated mice. To avoid any artifact introduced by the presence of the transgene, we analyzed nontransgenic BALB/c mice. Murine prolactin induced an expansion of mature B cells and a decrease in transitional B cells, mainly due to a decrease in the transitional type 1 (T1) B cells (CD21low/HSAhigh) (Figure 3, a and b). Of note, in placebo-treated mice the percent of transitional type 2 (T2) B cells (CD21high/HSAhigh) is lower than the percent of T1 cells; in prolactin-treated mice, there was no decrease in the percent of T2 cells as compared with the percent of T1 cells (Figure 3, a and b). These observations suggest that prolactin may decrease negative selection of immature B cells, which has been reported to occur within the transitional B cell population as T1 cells mature to T2 cells (54). Furthermore, prolactin induced an increase in both marginal zone (CD21high/CD23low) (Figure 3c) and follicular (CD21intermediate/HSAlow or CD2intermediate/CD23high) B cells (Figure 3, b and c, respectively). Ovine prolactin induced the same alteration in B cell subsets as murine prolactin (data not shown).

To identify which B cell subset was responsible for anti-DNA antibody production, we isolated follicular and marginal zone B cells from four placebo-treated mice and four R4A-γ2b BALB/c mice treated with ovine prolactin and enumerated spontaneously secreting anti-DNA B cells by ELISpot assay. There was a small increase in the number of IgG2b-producing anti-DNA antibody–secreting marginal zone B cells in prolactin-treated mice; however, there was a greatly expanded number of anti-dsDNA antibody–secreting cells in the follicular B cell population (Figure 3d).

Cellular and genetic studies of autoreactive B cells. To evaluate whether both the survival and the activation of autoreactive B cells observed in prolactin-treated mice requires the presence of T cells, we investigated the effect of prolactin on treatment of R4A-γ2b BALB/c mice deficient in CD4+ T cells (CD4 knockout mice). In the absence of CD4+ T cells, prolactin did not lead to increased serum titers of anti-DNA antibody (data not shown).

Figure 5
Gene expression. B cells from ovine prolactin- and placebo-treated mice were analyzed for cell surface and intracellular markers. (a) Bcl-2 expression was increased in B cells of prolactin-treated mice (P = 0.003). (b) CD40 expression was increased in B cells of prolactin-treated mice. (P = 0.008). MFI, mean fluorescence intensity.

Figure 6
Prolactin effects on B cells are genetically determined. Ten murine prolactin–treated and 10 placebo-treated R4A-γ2b C57Bl/6 mice were evaluated for serum anti-DNA reactivity, glomerular IgG deposits, and anti-DNA–secreting B cells. (a) There was no difference in the serum anti-DNA reactivity between prolactin- and placebo-treated mice. (b) Transgene-expressing B cells were equal in both prolactin- and placebo-treated mice. The total number of splenic B cells expressing γ2b was 2.12 ± 0.63 × 10^6 in placebo-treated mice, as compared with 2.23 ± 0.55 × 10^6 in prolactin-treated mice. (c) Prolactin-treated mice did not exhibit a higher number of B cells spontaneously secreting anti-DNA antibody or an increased responsiveness to anti-CD40 antibody and IL-4. Cells, anti-DNA antibody-secreting cells.
not shown). There was no expansion of the transgene-expressing population (Figure 4a), nor was there an increased number of spontaneously activated DNA-reactive B cells (Figure 4b). Thus, the effect of prolactin on both survival and activation of autoreactive B cells requires CD4+ T cells.

Prolactin-treated mice displayed an expansion of transgene-expressing B cells and a shift in the T1/T2 ratio similar to that which we have previously reported in estrogen-treated R4A-γ2b mice. To understand the molecular basis for these changes, we investigated the B cells from R4A-γ2b transgenic BALB/c mice for the expression of molecules important in the survival and activation of B cells that are known to be upregulated by estrogen, such as Bcl-2 (53), CD22, VCAM-1, and SHP-1 (55). Bcl-2 was upregulated by prolactin (Figure 5a), but no significant increase in the expression of the other molecules was observed in prolactin-treated mice (data not shown).

Because T cells were required for a prolactin-induced breakdown in B cell tolerance, we analyzed expression of the costimulatory molecules B7.1/B7.2 and CD40 on B cells after prolactin treatment. Prolactin increased the expression of CD40 on B cells (Figure 6) but did not significantly affect the expression of B7.1 or B7.2 (data not shown).

### Table 1

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**Discussion**

Clinical data and studies in animal models have implicated prolactin as a possible exacerbating factor in SLE. A significant number of patients with lupus have increased serum prolactin levels (29–31), and increased spontaneous secretion of prolactin has been demonstrated by peripheral-blood mononuclear cells of patients with lupus (41, 42). Hyperprolactinemia in lupus-prone mice accelerates disease activity and mortality (4, 5). Over the past several years, it has been appreciated that prolactin is an important component of neuroendocrine and immune interactions and functions as a regulator of the immune response, but its mechanisms of action at the cellular and molecular level have not been fully described.

We chose to study the effect of prolactin on the R4A-γ2b mouse. This mouse is well characterized and offers an opportunity to study survival and activation of different populations of autoreactive B cells. Using this model, we demonstrate that on a BALB/c genetic background, a twofold increase in serum prolactin breaks tolerance and induces a lupus-like illness. This increase is similar to that seen in patients with SLE and hyperprolactinemic mice. In R4A-γ2b BALB/c mice, treatment with prolactin induced an
Thus, in the absence of CD4+ T cells, there is no mature maintenance of the follicular B cell population (57). Some recent studies showing that T cells are required for the DNA-binding antibodies expressed germline V\(_{\kappa}\)1A light-chain genes. These observations demonstrate that prolactin rescues high-affinity autoreactive B cells, which are part of the naïve repertoire that normally undergo deletion.

We demonstrate in nontransgenic mice that prolactin alters B cell development and maturation, causing an expansion of the number of B cells with a mature phenotype and a decrease in the number of transitional B cells. In prolactin-treated, mice there is no decrease in the number of T2 B cells as compared with T1 B cells. Since it has been demonstrated that negative selection occurs at the T1 to T2 transition, this observation is consistent with a lack of negative selection of immature B cells (56). Prolactin treatment leads to an increase in both follicular and marginal zone B cell subsets, but the majority of autoreactive B cells develop as follicular B cells.

There are studies implicating both marginal zone and follicular B cells in the production of anti-DNA antibodies in murine lupus. Understanding which subset is critical for autoantibody production has potential therapeutic value. As each subset displays different requirements for activation, different interventions might be needed to block activation. Prolactin exerts its main autoimmune effects on the T cell–dependent follicular B cell subset. There was an absence of an autoimmune phenotype in prolactin-treated BALB/c mice that are deficient in CD4+ T cells. This is consistent with previous studies showing that T cells are required for the maintenance of the follicular B cell population (57). Thus, in the absence of CD4+ T cells, there is no mature population of anti-DNA B cells in prolactin-treated mice. Although treatment with prolactin also induces an expansion of the T-independent marginal zone B cell population, the contribution of this population to the prolactin-induced breakdown of tolerance is small.

Some recent studies suggest that marginal zone B cells develop when B cell receptor signaling is low, whereas follicular B cells develop under conditions of stronger B cell receptor signaling. Molecules that affect the strength of B cell receptor signaling such as Aiolos, Btk, and CD21 all alter B cell development, with a decrease in Aiolos or an increase in Btk or CD21 leading to the development of follicular cells (58). It has also been shown that Notch-RBP-J and Pyk-2 expression are critical for marginal zone B cell development, demonstrating that B cell receptor (BCR) signaling alone does not determine B cell fate (59).

Why prolactin causes autoreactive B cells to mature as follicular cells is not known; however, some of the changes we detect in gene expression may help explain the prolactin-induced altered B cell repertoire and the activation of autoreactive follicular B cells. Bcl-2 has been shown to enhance survival of immature autoreactive B cells in the bone marrow and to permit their transit to peripheral lymphoid organs (60). Prolactin has been reported to suppress apoptosis in hematopoietic cells and in cells in mammary and prostate glands, and recent studies in the Nb2 T cell line have shown that prolactin increases survival by stimulating the rapid expression of several anti-apoptotic genes, including bcl-2 (61). Our data demonstrate that prolactin can enhance Bcl-2 expression in B cells. This increase in Bcl-2 is likely to contribute to a decrease in negative selection or apoptosis of autoreactive B cells.

Prolactin has been shown to increase antigen presentation by dendritic cells (62). This observation has led to the speculation that prolactin might increase expression of CD40 (62). Here, we demonstrate that prolactin leads to increased expression of CD40 on B cells. It has previously been demonstrated that CD40 engagement can rescue transitional B cells from BCR-mediated apoptosis (63). We speculate that this rescue pathway is functional to permit autoreactive cells in prolactin-treated mice to reach maturity. It is interesting to note that CD40L expression has been demonstrated on B cells of patients with SLE (64), suggesting that enhanced costimulation is present. Furthermore, the finding that prolactin leads to increased CD40 expression is consistent with the requirement for T cells to be present for prolactin to modulate B cell selection and maturation. Because prolactin-induced survival and activation of anti-DNA B cells requires CD4+ T cells, costimulatory blockade may be a useful therapeutic tool for treatment of the prolactin-responsive subgroup of patients with lupus.

Although both estrogen and prolactin abrogate tolerance in R4A-\(\gamma_{2b}\) BALB/c mice, they lead to autoreactivity in different B cell subsets. This observation shows that estrogen does not exert its effect on the immune response by upregulation of prolactin. Prolactin-treated mice display autoreactive follicular B cells, whereas estrogen-treated mice display autoreactive marginal zone B cells. Differential features of gene expression help explain this difference. Several molecules that are implicated in B cell survival and activation that are elevated in estrogen-treated mice, such as CD22, VCAM-1, and SHP-1 (55), are not increased in B cells of prolactin-treated mice. It is reasonable to speculate that the increase in CD22 and SHP-1 induced by estrogen contributes to the greatly enhanced maturation of marginal zone B cells. Increased CD22 and SHP-1 would lead to reduced BCR signaling and the preferential development of a marginal zone phenotype. With the greater B cell receptor signaling that would occur with normal levels of CD22 and SHP-1, the follicular B cell phenotype would predominate. It is interesting to note that the
same anti-DNA B cells develop as follicular B cells in prolactin-treated mice and as marginal zone B cells in estrogen-treated mice (51). This observation confirms the observations of others that antigenic specificity alone does not determine the developmental pathway of a B cell (58, 59, 65, 66).

The same prolactin treatment that induced a lupus-like phenotype in RAA-γ2b BALB/c mice did not break tolerance in RAA-γ2b C57BL/6 mice, demonstrating the importance of the genetic background in determining the response to hormonal influences. Studies of animal models of SLE have identified more than 30 chromosomal regions containing genes that affect lupus susceptibility (67). Several of these loci map to overlapping regions of chromosomes 1, 5, 7, 11, 18, and 19 (68) and to the MHC locus on chromosome 17, but the majority of the detected loci affect lupus susceptibility (67). Several of these genes determine the response to hormonal influences.

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