Cutting Edge: Lupus Susceptibility Interval Sle3/5 Confers Responsiveness to Prolactin in C57BL/6 Mice

Elena Peeva, Juana Gonzalez, Ruthmarie Hicks and Betty Diamond

*J Immunol* 2006; 177:1401-1405; doi: 10.4049/jimmunol.177.3.1401

http://www.jimmunol.org/content/177/3/1401

---

**References**  This article cites 23 articles, 5 of which you can access for free at: http://www.jimmunol.org/content/177/3/1401.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Cutting Edge: Lupus Susceptibility Interval Sle3/5 Confers Responsiveness to Prolactin in C57BL/6 Mice

Elena Peeva,* Juana Gonzalez,* Ruthmarie Hicks,* and Betty Diamond†

Prolactin is of interest in the pathogenesis of systemic lupus erythematosus (SLE) because almost 25% of SLE patients display hyperprolactinemia, and serum prolactin correlates with disease activity in some patients. Furthermore, hyperprolactinemia causes early mortality in lupus-prone mice and induces a lupus-like phenotype in nonspontaneously autoimmune mice. We show here that the immunomodulatory effects of prolactin are genetically determined; hyperprolactinemia breaks B cell tolerance and causes a lupus-like serology in BALB/c mice expressing a transgene encoding the H chain of an anti-DNA Ab but not in lupus-like serology in BALB/c mice expressing a transgene encoding the H chain of an anti-DNA Ab but not in C57BL/6 transgenic mice. In C57BL/6 mice that express the H chain of an anti-DNA Ab and glomerular Ig depositions. The increase in cosstimulation due to prolactin-mediated up-regulation of both CD40 on B cells and CD40L on T cells would appear to play a central role in lupus induction in this model. The Journal of Immunology, 2006, 177: 1401–1405.

Systemic lupus erythematosus (SLE) is an autoimmune disease with an unclear etiology and a highly variable clinical presentation. It is characterized by a strong gender bias, with a female to male ratio of 9:1 during child-bearing age, suggesting that female sex hormones may play a crucial role in pathogenesis (1, 2). Genetic factors also affect the incidence of SLE, with African-Americans and Hispanics 2–4 times more likely to develop the disease than Caucasians (3).

Prolactin is a pituitary hormone best known for its lactogenic effect. Data accumulated over the past two decades have demonstrated that lymphocytes secrete prolactin and express receptors for it, pointing to a potential immunomodulatory role for the hormone (4, 5). Furthermore, both clinical studies in SLE patients and experimental studies in mice implicate prolactin in the development of autoimmunity (6) and in the pathogenesis of SLE (7, 8). Approximately 25% of SLE patients display some degree of hyperprolactinemia, usually mild to moderate (1), and some studies have found that the degree of hyperprolactinemia correlates with global disease activity (8). Hyperprolactinemia accelerates lupus activity and causes early mortality in lupus-prone mice (9). Doubling the serum prolactin level of BALB/c mice transgenic for a H chain of the pathogenic anti-DNA Ab R4A-γ2b breaks tolerance and induces a lupus-like phenotype. The same prolactin treatment has no effect on C57BL/6 mice bearing this transgene, indicating that the responsiveness of the immune system to prolactin is genetically determined (10).

In the study reported here, we demonstrate that genes responsible for this effect of prolactin on B cell tolerance lie within the lupus susceptibility interval Sle3/5. The Sle3/5 interval, derived from New Zealand mixed (NZM) 2410 lupus-prone mice, mediates an increase of the CD4:CD8 ratio in C57BL/6 mice and an increase in the number of activated CD4+ T cells (11), but prolactin is required to produce a lupus-like syndrome in this mouse strain. Prolactin up-regulates both CD40 and CD40L, which may be sufficient to mediate B cell rescue for apoptosis.

Materials and Methods

Mice

B6.NZW-Sle 3 (NZM 2410/Aeg) (Sle3/5 C57BL/6) mice were a gift from Dr. E. Wakeland (University of Texas Southwestern Medical Center, Dallas, TX). R4A-γ2b C57BL/6 mice were bred at the animal facility of the Albert Einstein College of Medicine (Bronx, NY). The R4A-γ2b C57BL/6 mice were generated by backcrossing the R4A-γ2b transgene onto the C57BL/6 background for >15 generations.

R4A-γ2b C57BL/6 mice were mated with Sle3/5 R4A-γ2b C57BL/6 mice. Eight- to 14-wk-old female Sle3/5 R4A-γ2b C57BL/6 mice were used in these studies.

Prolactin treatment

Pellets of placebo or prolactin (Innovative Research of America) that release 100 μg/day ovine prolactin (Sigma-Aldrich) were implanted s.c. and yielded serum prolactin levels of 68.3 ± 20.75 ng/ml, representing a mild increase over the normal serum prolactin levels (30.3 ± 19.7 ng/ml) (10) similar to the degree of hyperprolactinemia observed in patients with SLE. Placebo or prolactin treatment of the mice was maintained over a 5-wk period.

ELISA

Serum DNA-reactivity was evaluated by ELISA using Immunolon-2 plates (Dynex Technologies) coated with calf thymus DNA (Sigma-Aldrich). The assays were developed with an anti-IgG2b Ab (12).

1 This work was supported by the National Institutes of Health.
2 Address correspondence and reprint requests to Dr. Betty Diamond, Department of Medicine and Microbiology, Columbia University Medical Center, 1130 St. Nicholas Avenue, Audubon III Building, Room 916, New York, NY 10032. E-mail address: bd21376@columbia.edu
3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; NZM, New Zealand mixed.

Copyright © 2006 by The American Association of Immunologists, Inc.
ELISPOT assay

Splenocytes from placebo- or prolactin-treated mice, added in serial dilutions with dsDNA, were incubated at 37°C for 6 h. Biotin-conjugated goat anti-mouse IgG Ab (Southern Biotechnology Associates) at a 1/500 dilution was added, followed by streptavidin-conjugated alkaline phosphatase (Southern Biotechnology Associates) diluted at 1/1000. The plates were developed with 5-bromo-4-chloro-3-indolyl phosphate as substrate (Sigma-Aldrich). DNA-reactive ELISPOT assays were counted under a dissecting microscope.

Immunohistochemistry

Formalin-fixed, paraffin-embedded kidney sections from placebo- and prolactin-treated mice were deparaffinized in alcohol and then stained with biotinylated anti-mouse IgG Ab as previously described (12). The alkaline phosphatase ABC kit (Vector Laboratories) was used to develop the slides.

Flow cytometry

After RBC lysis with NH4Cl2, we performed surface staining of splenocytes for CD19, H9253, CD3, H9253, AA4.1, CD3, H9253, CD40, H9253, and CD40L with antibodies-conjugated to FITC, PE, allophycocyanin, or PE-Cy7 (BD Pharmingen) at 4°C for 30 min. The cells were then washed and fixed in 2% paraformaldehyde. Data was acquired by FACSCalibur flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Statistical analysis

Data were analyzed with standard statistical tests (mean value, SD, two-tailed Student’s t test, and Kruskal-Wallis nonparametric test).

Results

DNA reactivity in prolactin- and placebo-treated mice

The lupus susceptibility genetic interval Sle3/5 is associated with T cell hyperactivity (11), and induction of lupus in the R4A-γ2b BALB/c mouse model is T cell-dependent (8, 10). To determine whether the presence of Sle3/5 might allow prolactin to induce a lupus-like serology in R4A-γ2b C57BL/6 mice, we generated Sle3/5-bearing C57BL/6 mice that also carried the R4A-γ2b transgene. After treatment with prolactin for 5 wk, these mice had higher serum levels of anti-DNA Abs and an increased number of activated DNA-reactive B cells in comparison with placebo-treated mice as determined by ELISPOT assay (Fig. 1).

Lupus nephritis

Sle3/5-bearing, R4A-γ2b transgenic C57BL/6 mice treated with prolactin developed features of lupus nephritis, including proteinuria (Fig. 2A) and IgG deposition in the glomeruli (Fig. 2B), indicating that hyperprolactinemia enabled the production of high affinity nephritogenic anti-DNA Abs.

Thus, the R4A-γ2b transgene and the lupus susceptibility locus Sle3/5 do not by themselves induce lupus in C57BL/6 mice, but together they provide a susceptible genetic basis upon which other immunostimulatory factors such as prolactin can act to break B cell tolerance.

Transgene-expressing B cells

Like R4A-γ2b BALB/c mice and in contrast to R4A-γ2b C57BL/6 mice (10), Sle3/5 R4A-γ2b C57BL/6 mice responded to prolactin treatment with an expansion of the transgene-expressing B cell population (Fig. 3A). The increased number of transgene-expressing B cells was confirmed by immunohistochemical studies of the spleen. The increased number of γ2b-expressing B cells was primarily localized to the follicles (Fig. 3B).

B cell maturation

To evaluate the effects of hyperprolactinemia on B cell maturation in Sle3/5 R4A-γ2b transgenic C57BL/6 mice, we compared splenic B cell subsets in prolactin- and placebo-treated mice. Consistent with our previous data, prolactin-treated mice displayed a significantly decreased number of transitional T1 B cells (CD19+AA4.1+CD21−CD23−), leading to a significantly lower T1:T2 ratio than that present in placebo-treated mice (Fig. 4).

Increased serum prolactin levels did not significantly increase the numbers of mature marginal zone (CD19+AA4.1+CD21highCD23−) and follicular (CD19+AA4.1+CD21intermedCD23high) B cells (Fig. 4B), but did modestly bias the maturation pattern of γ2b-expressing B cells toward a follicular phenotype (Fig. 4C). The latter was confirmed by histology demonstrating an accumulation of transgene-expressing B cells in the splenic follicles of prolactin-treated mice (Fig. 3B). These observations indicate that hyperprolactinemia specifically induces the maturation of autoreactive B cells to the follicular phenotype. This finding is similar to our previous observations of prolactin-treated transgenic mice with the BALB/c background, where we have found that the follicular subset harbors the DNA-reactive B cells that spontaneously secrete autoantibodies.
A doubling of serum prolactin breaches B cell tolerance in R4A-γ2b BALB/c mice and induces a lupus-like syndrome. The increased serum prolactin alters B cell development, blocks negative selection of autoreactive specificities, and leads to an increased number of activated CD4+ T cells expressing CD40L (Fig. 5), indicating that heightened CD40-CD40L interactions could play a crucial role in the B cell hyperactivity in these mice.

**Discussion**

A doubling of serum prolactin alters B cell tolerance in R4A-γ2b BALB/c mice and induces a lupus-like syndrome. The increased serum prolactin alters B cell development, blocks negative selection of autoreactive specificities, and leads to an increased number of activated transgene-expressing B cells with a follicular phenotype. The effects of prolactin on B cells are accompanied by an up-regulation of Bcl-2 and CD40 expression (13). The same prolactin treatment has no effect on B cell development, survival, or activation in C57BL/6 mice bearing the same R4A-γ2b transgene (10). Hyperprolactinemia in these mice also does not alter the threshold for negative selection as assessed by the degree of apoptosis of isolated B cells on BCR engagement (E. Peeva and B. Diamond, unpublished data).

The prolactin-induced increase in CD40 expression may contribute to the development of a lupus-like syndrome by at least two mechanisms. First, ligation of CD40 may induce the expression of the anti-apoptotic proteins Bcl-xL and Bcl-2 (14) and, thus, may cause an increased survival of autoreactive transitional B cells. In addition, increased CD40 on B cells, along with a prolactin-mediated increase in the number of CD40L-expressing CD4+ T cells, may enhance activation and autoantibody production by mature B cells.

Genetic predisposition is a crucial factor in the susceptibility to human and murine lupus (15). Over 50 chromosomal regions containing genes responsible for lupus susceptibility or resistance have been identified (reviewed in Ref. 16). By linkage analysis of susceptibility to antinuclear Ab production and glomerulonephritis in lupus-prone NZM 2410 mice, Wakeland...
and colleagues (11) identified genetic intervals on several chromosomes that can induce specific autoimmune manifestations when transferred to nonlupus-prone mouse strains. Lupus susceptibility intervals on chromosome 1 (Sle1), chromosome 4 (Sle2), chromosome 7 (Sle3/5), and chromosome 17 (Sle4) were transferred onto a C57BL/6 genetic background producing congenic strains, each of which displayed an individual pattern of an autoimmune diathesis but not an autoimmune disease unilaterally. The anti-DNA-encoding transgene nor the lupus susceptibility interval also contains genes important for lymphocyte survival, signaling, and activation, including Bax, IL-4, CD22, CD37, TGF-β, and Bcl-3, all of which may play a role in the pathogenesis of lupus.

Prolactin down-regulates Bax in mammary glands (18) as well as in the Nb2 lymphoma cell line (19), and prolactin-mediated down-regulation of Bax may contribute to the prolactin-modulated survival of autoreactive B cells. IL-4 is a lysosomal l-amino acid oxidase (20) involved in peptide processing, and its altered expression may affect the MHC class II peptide repertoire (21). CD22 is a coreceptor which down-regulates BCR signaling and determines whether Ag-stimulated B cells undergo apoptosis or proliferation (22). CD22 knockout mice develop an autoimmune syndrome characterized by B cell hyperactivity with increased serum IgM and antinuclear Ab levels. In BALB/c mice, estrogen-up-regulated CD22 contributes to a breakdown of B cell tolerance and the development of a lupus-like syndrome (23), whereas tamoxifen-down-regulated CD22 appears to play a crucial role in the abrogation of estrogen-induced lupus (24). The importance of CD22 in the pathogenesis of human lupus has also been recognized, and a pilot clinical trial with anti-CD22 mAb demonstrated promising results (25). Although prolactin does not affect CD22 expression in BALB/c mice (10), the presence of other susceptibility genes and different background genes in Sle3/5 C57BL/6 mice may allow for certain epistatic relationships that can lead to a prolactin-mediated modulation of B cell activation via CD22. Finally, it is possible that the major contribution of Sle3 is to enhance the activation of dendritic cells.

Systemic lupus is a multifactorial disease, with genetic and hormonal factors implicated in its pathogenesis. This is reflected in the murine model used in our study, where neither the anti-DNA-encoding transgene nor the lupus susceptibility interval Sle3/5 was sufficient to induce lupus in a nonsusceptible mouse strain, but together they provided a genetic base upon which other immunostimulatory factors such as prolactin can act to break tolerance and produce disease.

FIGURE 4. Transgene-expressing B cells. A, Splenocytes were stained with fluorochrome-coupled Abs to CD19 and IgG2b. More y2b-expressing B cells were observed in prolactin-treated mice (n = 5) than in placebo-treated mice (n = 5) (p = 0.02). The absolute number of y2b+ B cells in the spleens of prolactin-treated mice was higher than that in mice treated with placebo (3.42 ± 0.64 × 10^6 vs. 2.1 ± 0.54 × 10^6). B, Representative plots depict y2b-expressing B cells in prolactin-treated mice compared with those in placebo-treated mice. C, R4A-y2b transgene-expressing B cells were identified by FITC-labeled anti-IgG2b Ab (green), and B cell follicles were identified with Rhodamine Red-X labeled anti-IgM Ab (red). Placebo-treated mice displayed fewer y2b-expressing B cells than prolactin-treated mice. In placebo-treated mice, the transgene-expressing B cells were present mainly in the red pulp and to a lesser degree in the T cell zone, whereas in prolactin-treated mice they were predominantly localized in the follicles.

FIGURE 5. CD40 and CD40L expression. Splenocytes from prolactin- and placebo-treated mice were surface stained for CD40 and CD40L. A, Compared with B cells from placebo-treated mice (n = 5), B cells from prolactin-treated mice (n = 5) demonstrated an increased expression of CD40 (p = 0.003). MFI, mean fluorescence intensity. B, Prolactin-treated mice displayed increased number of activated CD40+CD4+ T cells (p = 0.02).
Acknowledgments
We thank Ward Wakeland for the Sle3/5 mice, Yi Bao for staining the splenic sections, and Sylvia Jones for help with preparation of the manuscript.

Disclosures
The authors have no financial conflict of interest.

References