Published in final edited form as:

Genet Med. 2008 December ; 5(4): 423–433. doi:10.1016/j.genm.2008.10.001.

Estrogen and Progesterone Affect Responses to Malaria Infection in Female C57BL/6 Mice

Pamela W. Klein, B.A.* , Judith D. Easterbrook, Ph.D., Erin N. Lalime, B.S., and Sabra L. Klein, Ph.D.

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Abstract

Background—Previous data from our laboratory suggest that gonadally intact C57BL/6 male mice are more likely than their female counterparts to die from *Plasmodium chabaudi* infection, to recover more slowly from weight loss and hematocrit loss, and to have reduced interferon-gamma (IFN- γ) and interleukin (IL)-10 responses. Removal of the ovaries, and hence, the primary production of sex steroids in females, reverses these differences.

Objective—We hypothesized that sex differences in response to *P. chabaudi* may be mediated by differential synthesis of IFN- γ and IL-10 that is influenced by estrogen, progesterone, or both.

Methods—C57BL/6 female mice (n = 200; n = 10/time point/treatment/experiment) were ovariectomized (OVX) and implanted with either a 21-day controlled-release pellet containing 0.1 mg of 17 β -estradiol (E₂), 10 mg of progesterone (P₄), 0.1 mg of E₂ plus 10 mg of P₄, or cholesterol (placebo). Females were inoculated with 10⁶ *P. chabaudi*-infected erythrocytes. Body mass, body temperature, hematocrit, parasitemia, cytokine production, and antibody responses were monitored 0, 3, 5, 7, 10, 14, and 21 days postinoculation.

Results—Administration of E₂, either alone or in combination with P₄, mitigated infection-induced weight loss, hematocrit loss, and hypothermia, as compared with females receiving placebo pellets ($P < 0.05$ in each case). Hormone treatment did not affect levels of parasitemia. Females administered E₂ alone or in combination with P₄ produced 50–150 times more IFN- γ and IL-10 during peak parasitemia than did females implanted with pellets containing either P₄ alone or placebo ($P < 0.05$ in each case). Exposure to E₂, either alone or in combination with P₄, increased anti-*P. chabaudi* immunoglobulin G (IgG1) responses and the ratio of IgG1 to IgG2c ($P < 0.05$ in each case).

Conclusion—This animal study suggests that physiological levels of estrogen, rather than progesterone, enhance immunity and, possibly, protect females from disease symptoms during malaria infection.

Keywords

estrogen; interferon- γ ; interleukin-10; malaria; progesterone; sex difference; sex steroid

INTRODUCTION

Among humans and animals, the prevalence and severity of parasitic infections has been reported to be greater in males than in females.¹ One genus of protozoan parasites that causes a pronounced sexual dimorphism in vertebrate hosts is *Plasmodium*. Most studies of malaria in human populations have not distinguished between the responses of males and females and, thus, the prevalence of sex differences may be underreported.² A few epidemiologic studies have, however, established the presence of sex differences in *Plasmodium* infection among humans. The incidence and intensity of *Plasmodium falciparum* infection are reportedly higher in men than in women.^{3–6} Men and women also differ in disease manifestations following infection.^{7–9} Among Ghanaian schoolchildren, although the prevalence of *P. falciparum* infection did not differ between the sexes, parasite density was 2-fold higher around puberty (ages 8–16 years) in boys (549.4 parasites/ μ L of blood) than in girls (243.4 parasites/ μ L of blood), suggesting that circulating sex steroids may influence this outcome.⁵ Sex differences in response to malaria infection have been reported among both adults and children^{3–9}, but little is known about the mechanisms mediating these sex differences or whether these differences affect responses to drug treatments or vaccines.

Clinical and epidemiologic studies have established the presence of sex differences in *Plasmodium* infection among humans^{3–9}; animal models have been complementary for characterizing the mechanisms that underlie sex differences in response to *Plasmodium* infection. Studies of rodent malaria infection reported that males were 3 to 6 times more likely to die after blood-stage malaria infection than were females.^{10–12} Castration of males reduced, whereas exogenous administration of testosterone increased, mortality after infection with *Plasmodium chabaudi* or *Plasmodium berghei* in mice.^{12–14} In addition to increased mortality rates, male mice recovered more slowly from *P. chabaudi*-induced weight loss, anemia, and hypothermia than did females.^{14, 15} The immunosuppressive effects of testosterone may underlie increased susceptibility to *Plasmodium* infections in males compared with females. Exposure of adult female mice to high doses of testosterone reduced antibody production, decreased major histocompatibility complex (MHC) class II cells in the spleen, increased CD8⁺ T cells in the spleen, and reduced the expression of malaria-responsive genes in the liver, but did not affect cytokine production.^{10, 16} Recent data from our laboratory illustrate that gonadally intact male mice have significantly reduced interferon gamma (IFN- γ)-associated gene expression, IFN- γ production, and regulatory T-cell gene expression during peak parasitemia and produce less antibody during the recovery phase of infection than do females.¹⁴ Removal of the ovaries and, hence, the primary production of estrogens and progesterone (P₄) in female mice significantly reduced these responses, illustrating that ovarian hormones may modulate proinflammatory, regulatory, and humoral responses to malaria.

Examination of the effects of 17 β -estradiol (E₂) and P₄ on the course of malaria infection have yielded contradictory results. Studies utilizing *P. chabaudi* or *P. berghei* infection of female mice and *Plasmodium knowlesi* infection of female rhesus monkeys revealed that supraphysiological doses of either E₂ or P₄ increased peak parasitemia and parasite recrudescence and reduced survival,^{17–20} possibly through suppression of CD4⁺ T cells.¹⁷ Pregnancy-associated changes in cell-mediated immune responses and increased susceptibility to *Plasmodium* infections have been attributed to hormonal changes that occur during pregnancy, including increased concentrations of estrogens, progestins, and glucocorticoids.^{21–23} Although excessively high doses of E₂ (eg, during pregnancy) may increase susceptibility to infection, studies have illustrated that E₂, at physiological doses, enhanced innate, cell-mediated, and humoral immunity.^{24–27} Conversely, P₄ often suppresses immune responses, including the activity of dendritic cells and T cells^{28–30}; thus, P₄ may serve to regulate E₂-enhanced immune responses to prevent the overproduction of immunologic proteins and the development of immunopathology. Whether physiological doses of E₂, alone or in combination with P₄, can enhance “protective immunity” and reduce morbidity during nonlethal malaria infection has not been documented and was the goal of the present study.

MATERIALS AND METHODS

Animals

Adult (>60 days of age) female (n = 200; 10/time point/treatment/experiment) C57BL/6 mice were purchased from the National Cancer Institute (Bethesda, Maryland). All animals were housed 5 to a cage in a microisolator room and maintained on a constant light:dark 16:8-hour cycle. Food and sterile tap water were available ad libitum. The Johns Hopkins Animal Care and Use Committee (protocol no. MO04H532) and the Johns Hopkins Office of Health, Safety, and Environment (protocol no. P0303310202) approved all procedures described.

Infection

P. chabaudi chabaudi AS parasites were kindly provided by Dr. Mary M. Stevenson (McGill University, Montreal, Canada) and were maintained in donor BALB/c mice by weekly passage from frozen stock cultures. Parasites were passaged 2 to 3 times before use in experimental animals. All experimental animals received an intraperitoneal inoculation of 10⁶ *P. chabaudi chabaudi* AS-infected erythrocytes.

Procedures

Females were bilaterally ovariectomized (OVX) using ketamine (80 mg/kg body mass)–xylazine (8 mg/kg body mass) anesthesia (Phoenix Pharmaceutical Inc., St. Joseph, Missouri) and given 2 to 3 weeks to recover from surgery. At the time of infection, females were implanted with a 21-day controlled-release pellet containing 0.1 mg of E₂, 10 mg of P₄, 0.1 mg of E₂ plus 10 mg of P₄, or cholesterol (placebo) (Innovative Research of America, Sarasota, Florida). After infection, animals were killed 3, 7, or 21 days postinoculation (pi) to examine responses prior to peak parasitemia (day 3 pi), during peak parasitemia (day 7 pi), and during the recovery phase of infection (day 21 pi). At days 5, 10,

and 14 pi, blood was collected from the saphenous vein to assess anemia, parasitemia, and anti-*P. chabaudi* antibody responses, and body mass and rectal temperature were recorded. Spleens were dissected and weighed, and splenocytes were isolated and used to measure protein production. Samples also were collected from uninfected female mice (designated day 0 pi) in each treatment group.

Parasitemia—Thin blood smears were prepared, fixed with methanol, and stained with a 1:10 dilution of Giemsa stain (Sigma-Aldrich Corporation, St. Louis, Missouri) in 1X phosphate buffer as described previously.¹⁴ Parasites were visualized under a 100X oil immersion lens, and parasitemia was calculated by counting the number of parasites per total number of erythrocytes in a minimum of 3 random fields.

Hematocrit—Blood was collected into heparinized microhematocrit tubes, which were plugged with clay and centrifuged for 15 minutes at 1200 rpm. Packed red blood cell (RBC) volume was measured relative to total blood volume.

Body Temperature—To monitor body temperature, animals were briefly restrained in a modified 50-mL conical tube and rectal temperature was measured within 5 seconds (Physitemp Instruments, Inc., Clifton, New Jersey).

Splenocyte Isolation—Spleens were placed in sterile RPMI-1640 medium (Mediatech, Inc., Manassas, Virginia), and splenocytes were recovered by pressing the whole spleen through a 100-mm nylon cell strainer. Separated cells were suspended in additional RPMI-1640, and erythrocytes were lysed with ACK lysis buffer (Invitrogen Corporation, Carlsbad, California). Cells were washed twice, resuspended in 10 mL of PBS, and splenocyte counts and viability were determined using a hemacytometer and trypan blue exclusion.

Cytokine Enzyme-Linked Immunosorbent Assays (ELISAs)—Viable splenocytes were adjusted to 5×10^6 cells/mL in supplemented culture medium (RPMI-1640 with 25 mM HEPES, 10% fetal bovine serum, 2 mM L-glutamine, 1% penicillin-streptomycin, and 0.2 mM 2-mercaptoethanol). Plates were incubated at 37°C with 5% CO₂ for 24 hours. IFN- γ and interleukin (IL)-10 concentrations were assayed by ELISA using the manufacturer's protocols for the OptEIA IFN- γ and IL-10 kits (BD Pharmingen, Inc., San Diego, California).

Hormone Enzyme Immunoassays (EIAs)—E₂ and P₄ were extracted using a standard ether extraction protocol, and samples were resuspended in the manufacturer-provided assay buffer (1:10 final dilution; Cayman Chemical Company, Ann Arbor, Michigan). Steroid concentrations were measured according to the manufacturer's protocol. Extraction efficiency was measured using a spiked standard and was used to correct for steroid loss during extraction. For the estradiol EIA, sensitivity was 80% at 19 pg/mL; for the progesterone EIA, sensitivity was 80% at 10 pg/mL.

Anti-*P. chabaudi* ELISA

Microtiter plates were coated overnight at 4°C in carbonate buffer with 5 µg/mL of *P. chabaudi* antigen for immunoglobulin G (IgG) or 20 µg/mL of *P. chabaudi* antigen for IgG1 and IgG2c as described previously.¹⁴ Plates were blocked, and plasma samples, as well as positive and negative control samples, were diluted as described previously. Secondary antibody (horseradish peroxidase [HRP]-goat anti-mouse IgG [Cayman Chemical], HRP-goat anti-mouse IgG1 (Southern Biotechnology Associates Inc., Birmingham, Alabama), and HRP-goat anti-mouse IgG2c (Southern Biotechnology Associates) were added, and reactions were visualized using a tetramethylbenzidine substrate (BD Pharmingen) and terminated after 30 minutes by adding 2 N H₂SO₄. The optical density was measured at 450 nm, and samples were expressed as a percentage of the positive control run on the same microtiter plate.

Statistical Analyses

Parasitemia, anemia, rectal temperature, and body mass changes were analyzed using mixed analyses of variance (ANOVAs) with 1 within-subjects variable (days pi) and 1 between-subjects variable (treatment group). Cytokine concentrations were assessed using 2-way ANOVAs, and antibody responses were analyzed using 1-way ANOVAs. Significant interactions were further analyzed using planned comparisons or the Tukey test for pairwise multiple comparisons. Mean differences were considered statistically significant if *P* was <0.05.

RESULTS

E₂, alone or in combination with P₄, reduced morbidity during *P. chabaudi* infection in OVX female mice

OVX reduced both E₂ and P₄ concentrations to nondetectable levels. Implantation of E₂ pellets, either alone (259 ± 58 pg/mL) or in combination with P₄ (662 ± 178 pg/mL), significantly increased circulating E₂ concentrations when compared with concentrations in females implanted with either placebo (31 ± 8 pg/mL) or P₄ alone (59 ± 13 pg/mL) (*P* < 0.001). Administration of P₄ pellets, either alone (51.6 ± 22.8 ng/mL) or with E₂ (56.1 ± 18.8 ng/mL), significantly increased concentrations of P₄ when compared with concentrations in females implanted with either placebo (1.3 ± 0.3 ng/mL) or E₂ alone (1.0 ± 0.2 ng/mL) (*P* < 0.001).

Body mass, proportion of RBCs, and rectal temperature were monitored at several time points during malaria infection. After inoculation with *P. chabaudi*, body mass, rectal temperature, and proportions of RBCs decreased significantly through day 10 pi and returned to baseline by day 21 pi for all animals (*P* < 0.001 in each case) (Figures 1A–C). Females treated with E₂, alone or in combination with P₄, lost less weight and returned to baseline weight faster than females treated with placebo or P₄ alone (*P* < 0.001) (Figure 1A). Development of hypothermia was marginally reduced in females treated with E₂ or E₂ plus P₄ compared with females treated with placebo or P₄ alone (*P* = NS) (Figure 1B). Females treated with either E₂ or E₂ plus P₄ presented with a less-severe decrease in hematocrit 10 days pi than did females exposed to placebo or P₄ alone (*P* < 0.002) (Figure

1C). Levels of parasitemia peaked in all animals at 7 days pi ($P < 0.001$) (Figure 1D). There was, however, no significant effect of hormone manipulation on either peak parasitemia or clearance of *P. chabaudi* parasites by 21 days pi ($P = \text{NS}$).

During peak parasitemia, E₂ increased IFN- γ and IL-10 production

In the present study, production of IFN- γ peaked at day 7 pi for all groups of females, regardless of hormone treatment ($P < 0.001$) (Figure 2A). Females treated with E₂ and P₄ produced more IFN- γ during peak parasitemia than did females treated with placebo or P₄ alone ($P < 0.001$). Females treated with E₂ alone produced marginally higher amounts of IFN- γ than did placebo or P₄-treated females ($P = \text{NS}$).

Production of IL-10 was highest during peak parasitemia for all groups of females, regardless of hormone treatment ($P < 0.001$) (Figure 2B). Females treated with E₂ alone and, to a lesser extent, with E₂ plus P₄ had increased production of IL-10 7 days pi compared with females implanted with placebo or P₄ alone ($P < 0.001$).

During the recovery phase of infection, E₂, alone or in combination with P₄, enhanced anti-*P. chabaudi* IgG1 responses and the ratio of IgG1:IgG2c produced

In addition to cell-mediated effector mechanisms, humoral responses are required to eliminate the erythrocytic stage of *Plasmodium* parasites. To test the hypothesis that E₂ alone or in combination with P₄ enhances production of antibody against *P. chabaudi*, blood samples collected 21 days pi were used to measure anti-*P. chabaudi* IgG, IgG2c, and IgG1. Neither anti-*P. chabaudi* total IgG nor anti-*P. chabaudi* IgG2c responses differed significantly among the treatment groups ($P = \text{NS}$) (Figures 3A and 3B). Exposure to E₂, either alone or in combination with P₄, increased anti-*P. chabaudi* IgG1 responses and the ratio of IgG1 to IgG2c in females ($P < 0.05$ in each case) (Figures 3C and 3D).

DISCUSSION

Sex-specific susceptibility to malaria infection is widely reported in mouse models.^{10–12} Thus, malaria infection of mice provides us with a system for manipulating and examining the direct effects of sex steroids on immune responses to infection. Infection of C57BL/6 mice with non-lethal *P. chabaudi* induces weight loss, hypothermia, and a transient reduction of hematocrit.¹⁵ In the present study, administration of E₂, either alone or in combination with P₄, significantly reduced infection-induced weight loss, hematocrit loss, and hypothermia. Hormone treatment did not affect levels of parasitemia. These data suggest that E₂ drives the female-typic physiological response to malaria, but has no effect on the ability of the parasite to infect and replicate in RBCs.

Sex differences in susceptibility to protozoan infections, such as malaria, may reflect an immunologic dimorphism, in which females often mount more robust immune responses than do males.^{1, 31} The prevailing hypothesis for heightened immunologic responsiveness in females compared with males is that sex hormones directly influence immune function. Receptors for sex steroids, including androgen, estrogen, and progesterone receptors, are expressed on many immune cells.¹ To examine sex steroid-mediated effects on immunity, in vitro studies often are conducted in which isolated cell populations are exposed to varying

concentrations of sex hormones.³² Most in vitro studies involve examination of the effects of estrogens on immune cells, including lymphocytes, macrophages, and dendritic cells, with fewer studies considering the immunomodulatory role of P₄.^{28, 30} Although in vitro studies have yielded a plethora of data pertaining to the direct effects of sex steroids on immunity, there are notable differences in the effects of sex steroids on cell populations in vitro and in vivo.²⁷ Thus, studies in which sex hormones are manipulated in vivo and subsequent responses are examined during infection are important for accurately examining the dynamic interactions that occur between the endocrine and immune systems. Also, the extent to which ovarian sex steroids, specifically estrogens and P₄, act alone or in combination to affect responses to infection is rarely considered.

Protection against malaria involves complex interactions between innate and adaptive immune effector responses that limit peak parasitemia and promote clearance of parasites.³³ Production of IFN- γ , in particular, contributes to the control of blood-stage malaria parasites in both humans and mice. In humans, IFN- γ production is elevated during the acute asexual phase of blood-stage *P. falciparum* infection.^{34–36} In mice, in vivo depletion of natural killer cells results in severe disease and high peak parasitemia that is mediated by reduced production of IFN- γ during peak parasitemia as opposed to cytotoxic activity.³⁷ During *P. chabaudi* infection, females produce more IFN- γ than do males during peak parasitemia.¹⁴ Elimination of IFN- γ abolishes the sexual dimorphism in morbidity and mortality by increasing susceptibility of IFN- γ -deficient females to infection when compared with wild-type females.¹⁴ Exposure to E₂ enhances production of IFN- γ ^{27, 38, 39}; a process likely mediated by the presence of estrogen response elements in the promoter region of the *Ifng* gene.⁴⁰ In the present study, combined exposure to E₂ and P₄ led to the greatest increase in production of IFN- γ during peak parasitemia. Whether the increased production of IFN- γ in E₂ plus P₄ females reflects their higher circulating concentration of E₂ compared with females treated with E₂ alone is a plausible hypothesis. Progesterone alone reduced production of IFN- γ compared with treatment with either E₂ alone or in combination with P₄, as has been reported previously using lymphocytes isolated from women and exposed to P₄ in vitro.^{41, 42} Future studies must continue to consider the role of P₄ in mediating IFN- γ production, because the combined treatment with E₂ and P₄ led to the greatest increase in IFN- γ production.

The induction of regulatory responses is essential to control early inflammatory responses and reduce development of host-mediated pathology. Two cytokines that mediate regulatory T-cell activity are transforming growth factor- β (TGF- β) and IL-10.⁴³ In mice, both antibody neutralization of TGF- β in *P. chabaudi*-resistant mice and examination of responses to murine malaria in IL-10-deficient mice reveal that regulatory responses are instrumental for downregulating proinflammatory responses and extending survival following infection.^{44, 45} After inoculation with *P. chabaudi*, IL-10 expression is elevated in females compared with males during peak parasitemia,¹⁴ and removal of IL-10 is more detrimental to female than to male mice.⁴⁶ In the present study, P₄ suppressed production of IL-10. Conversely, administration of E₂ alone increased production of IL-10 during peak parasitemia, which supports previous observations that E₂ augmented the expansion of regulatory T cells in mice.⁴⁷ Females exposed to E₂ either alone or in combination with P₄ collectively produced

the highest concentrations of IFN- γ and IL-10 and had the fastest recovery from infection, suggesting that the immunomodulatory effects of E₂ may protect females from symptoms of disease during malaria infection.

Antibody responses are important for clearance of malaria parasites from circulation.³³ Previous data from our laboratory indicate that anti-*P. chabaudi* IgG and IgG1 (ie, helper T cell type 2 (Th2)-mediated antibody production), but not IgG2c (ie, Th1-mediated antibody production), responses are higher among gonadally intact females than intact males 14 to 21 days pi, and ovariectomy of female mice reverses this sex difference by 21 days pi.¹⁴ In the present study, manipulation of ovarian steroids, however, did not affect the ability of females to clear parasites from circulation; all females, regardless of their sex hormone concentrations, cleared parasites by 21 days pi. Exposure to E₂, either alone or in combination with P₄, increased anti-*P. chabaudi* IgG1 responses and the ratio of IgG1 to IgG2c in females, which supports previous findings that estrogens potentiated antibody production by B-cells.⁴⁸ Deletion of B cells in μ MT mice, however, does not abolish the sexual dimorphism in response to *P. chabaudi* infection, suggesting that B cells may not be critical mediators of sex differences in malaria infection.¹⁴

The balance between generating protective inflammatory responses to clear malaria parasites and regulation of anti-malaria immunity to prevent pathology is critical for survival from malaria infection.⁴³ In the present study, female mice administered E₂ either alone or in combination with P₄ experienced reduced morbidity and produced higher concentrations of proinflammatory and regulatory cytokines than did females administered placebo or P₄ alone. Thus, physiological levels of estrogen may regulate immunity and, possibly, protect female mice during malaria infection. Future studies should continue to examine the effects of ovarian steroids, at physiological and pregnancy-associated levels, on additional immune responses during malaria infection. Although there are notable differences in responses to malaria parasites between humans and mice, the data in the present study emphasize that consideration should be paid to sex differences and the effects of endogenous and exogenous hormones on immune responses during malaria infection in humans.

Acknowledgments

The authors thank Jenifer Kaplan for technical assistance. Financial support for this study was provided by the Johns Hopkins Malaria Research Institute and National Institutes of Health grant R01 AI054995.

REFERENCES

1. Klein SL. Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunol.* 2004; 26:247–264. [PubMed: 15541029]
2. Allotey, P.; Gyapong, M. The gender agenda in the control of tropical diseases: A review of current evidence. Geneva, Switzerland: World Health Organization Special Programme for Research; 2005.
3. Weise VH-J. Imported cases of malaria into the Federal Republic of Germany and West Berlin with particular emphasis upon the last 5 years, 1973–1977 [in German]. *Bundesgesundheitsblatt.* 1979; 22:1–7.
4. Wildling E, Winkler S, Kremsner PG, et al. Malaria epidemiology in the province of Moyen Ogoov, Gabon. *Trop Med Parasitol.* 1995; 46:77–82. [PubMed: 8525289]

5. Landgraf B, Kollaritsch H, Wiedermann G, Wernsdorfer WH. Parasite density of *Plasmodium falciparum* malaria in Ghanaian schoolchildren: Evidence for influence of sex hormones? *Trans R Soc Trop Med Hyg.* 1994; 88:73–74. [PubMed: 8154009]
6. Molineaux, L.; Gramiccia, G. The Garki Project: Research on the Epidemiology and Control of Malaria in the Sudan Savannah of West Africa. Geneva, Switzerland: World Health Organization; 1980.
7. Brabin BJ. An analysis of malaria parasite rates in infants, 40 years after MacDonald. *Trop Dis Bull.* 1990; 87:R1–R21.
8. Brabin BJ, Brabin L, Crane G, et al. Two populations of women with high and low spleen rates living in the same area of Madang, Papua New Guinea, demonstrate different immune responses to malaria. *Trans R Soc Trop Med Hyg.* 1989; 83:577–583. [PubMed: 2694494]
9. Morrow RH, Kisuule A, Pike MC, Smith PG. Burkitt's lymphoma in the Mengo Districts of Uganda: Epidemiologic features and their relationship to malaria. *J Natl Cancer Inst.* 1976; 56:479–483. [PubMed: 1255780]
10. Benten WP, Ulrich P, Kühn-Velten WN, et al. Testosterone-induced susceptibility to *Plasmodium chabaudi* malaria: Persistence after withdrawal of testosterone. *J Endocrinol.* 1997; 153:275–281. [PubMed: 9166117]
11. Zhang Z, Chen L, Saito S, et al. Possible modulation by male sex hormone of Th1/Th2 function in protection against *Plasmodium chabaudi* chabaudi AS infection in mice. *Exp Parasitol.* 2000; 96:121–129. [PubMed: 11162362]
12. Wunderlich F, Marinovski P, Benten WP, et al. Testosterone and other gonadal factors restrict the efficacy of genes controlling resistance to *Plasmodium chabaudi* malaria. *Parasite Immunol.* 1991; 13:357–367. [PubMed: 1923561]
13. Kamis AB, Ibrahim JB. Effects of testosterone on blood leukocytes in *Plasmodium berghei*-infected mice. *Parasitol Res.* 1989; 75:611–613. [PubMed: 2671986]
14. Cernetich A, Garver LS, Jedlicka AE, et al. Involvement of gonadal steroids and gamma interferon in sex differences in response to blood-stage malaria infection. *Infect Immun.* 2006; 74:3190–3203. [PubMed: 16714546]
15. Sanni LA, Fonseca LF, Langhorne J. Mouse models for erythrocytic-stage malaria. *Methods Mol Med.* 2002; 72:57–76. [PubMed: 12125155]
16. Krücken J, Dkhil MA, Braun JV, et al. Testosterone suppresses protective responses of the liver to blood-stage malaria. *Infect Immun.* 2005; 73:436–443. [PubMed: 15618182]
17. Benten WP, Wunderlich F, Herrmann R, Kühn-Velten WN. Testosterone-induced compared with oestradiol-induced immunosuppression against *Plasmodium chabaudi* malaria. *J Endocrinol.* 1993; 139:487–494. [PubMed: 8133215]
18. Kamis AB. *Plasmodium berghei* infection in mice: Effects of estradiol. *Mosq Borne Dis Bull.* 1991; 8:45–47.
19. Libonati RM, Cunha MG, Souza JM, et al. Estradiol, but not dehydroepiandrosterone, decreases parasitemia and increases the incidence of cerebral malaria and the mortality in *Plasmodium berghei* ANKA-infected CBA mice. *Neuroimmunomodulation.* 2006; 13:28–35. [PubMed: 16699290]
20. Dutta GP, Kamboj KK. Influence of progesterone and estrogen administration on the recrudescence patterns of *Plasmodium knowlesi* infection in female rhesus monkeys (*Macaca mulatta*) following initial subcurative chloroquine therapy. *Indian J Malariol.* 1984; 21:79–88. [PubMed: 6536543]
21. Vleugels MP, Brabin B, Eling WM, de Graaf R. Cortisol and *Plasmodium falciparum* infection in pregnant women in Kenya. *Trans R Soc Trop Med Hyg.* 1989; 83:173–177. [PubMed: 2692225]
22. Van Zon AA, Eling WM, Hermesen CC, et al. Malarial immunity in pregnant mice, in relation to total and unbound plasma corticosterone. *Bull Soc Pathol Exot Filiales.* 1983; 76:493–502. [PubMed: 6370474]
23. Van Zon AA, Termaat RM, Schetters TP, Eling WM. *Plasmodium berghei*: Reduction of the mouse's specific lymphoproliferative response in relation to corticosterone and pregnancy. *Exp Parasitol.* 1986; 62:71–78. [PubMed: 3522263]

24. Verthelyi D. Female's heightened immune status: Estrogen, T cells, and inducible nitric oxide synthase in the balance. *Endocrinology*. 2006; 147:659–661. [PubMed: 16418430]
25. Karpuzoglu-Sahin E, Hissong BD, Ansar Ahmed S. Interferon-gamma levels are upregulated by 17-beta-estradiol and diethylstilbestrol. *J Reprod Immunol*. 2001; 52:113–127. [PubMed: 11600182]
26. Karpuzoglu E, Fenaux JB, Phillips RA, et al. Estrogen up-regulates inducible nitric oxide synthase, nitric oxide, and cyclooxygenase-2 in splenocytes activated with T cell stimulants: Role of interferon-gamma. *Endocrinology*. 2006; 147:662–671. [PubMed: 16293660]
27. Siracusa MC, Overstreet MG, Housseau F, et al. 17beta-estradiol alters the activity of conventional and IFN-producing killer dendritic cells. *J Immunol*. 2008; 180:1423–1431. [PubMed: 18209037]
28. Butts CL, Shukair SA, Duncan KM, et al. Progesterone inhibits mature rat dendritic cells in a receptor-mediated fashion. *Int Immunol*. 2007; 19:287–296. [PubMed: 17289656]
29. Miyaura H, Iwata M. Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. *J Immunol*. 2002; 168:1087–1094. [PubMed: 11801642]
30. Hughes GC, Thomas S, Li C, et al. Cutting edge: Progesterone regulates IFN-alpha production by plasmacytoid dendritic cells. *J Immunol*. 2008; 180:2029–2033. [PubMed: 18250406]
31. Roberts CW, Walker W, Alexander J. Sex-associated hormones and immunity to protozoan parasites. *Clin Microbiol Rev*. 2001; 14:476–488. [PubMed: 11432809]
32. Hughes GC, Clark EA. Regulation of dendritic cells by female sex steroids: Relevance to immunity and autoimmunity. *Autoimmunity*. 2007; 40:470–481. [PubMed: 17729041]
33. Stevenson MM, Riley EM. Innate immunity to malaria. *Nat Rev Immunol*. 2004; 4:169–180. [PubMed: 15039754]
34. Artavanis-Tsakonas K, Eleme K, McQueen KL, et al. Activation of a subset of human NK cells upon contact with *Plasmodium falciparum*-infected erythrocytes. *J Immunol*. 2003; 171:5396–5405. [PubMed: 14607943]
35. Artavanis-Tsakonas K, Riley EM. Innate immune response to malaria: Rapid induction of IFN-gamma from human NK cells by live *Plasmodium falciparum*-infected erythrocytes. *J Immunol*. 2002; 169:2956–2963. [PubMed: 12218109]
36. Korbel DS, Finney OC, Riley EM. Natural killer cells and innate immunity to protozoan pathogens. *Int J Parasitol*. 2004; 34:1517–1528. [PubMed: 15582528]
37. Mohan K, Moulin P, Stevenson MM. Natural killer cell cytokine production, not cytotoxicity, contributes to resistance against blood-stage *Plasmodium chabaudi* AS infection. *J Immunol*. 1997; 159:4990–4998. [PubMed: 9366426]
38. Nakaya M, Tachibana H, Yamada K. Effect of estrogens on the interferon-gamma producing cell population of mouse splenocytes. *Biosci Biotechnol Biochem*. 2006; 70:47–53. [PubMed: 16428820]
39. Sorachi K, Kumagai S, Sugita M, et al. Enhancing effect of 17 beta-estradiol on human NK cell activity. *Immunol Lett*. 1993; 36:31–35. [PubMed: 8344714]
40. Fox HS, Bond BL, Parslow TG. Estrogen regulates the IFN-gamma promoter. *J Immunol*. 1991; 146:4362–4367. [PubMed: 1904081]
41. Choi BC, Polgar K, Xiao L, Hill JA. Progesterone inhibits in-vitro embryotoxic Th1 cytokine production to trophoblast in women with recurrent pregnancy loss. *Hum Reprod*. 2000; 15(Suppl 1):46–59. [PubMed: 10928418]
42. Raghupathy R, Al Mutawa E, Makhseed M, et al. Modulation of cytokine production by dydrogesterone in lymphocytes from women with recurrent miscarriage. *BJOG*. 2005; 112:1096–1101. [published correction appears in *BJOG*. 2005; 112: 1585]. [PubMed: 16045524]
43. Riley EM, Wahl S, Perkins DJ, Schofield L. Regulating immunity to malaria. *Parasite Immunol*. 2006; 28:35–49. [PubMed: 16438675]
44. Li C, Sanni LA, Omer F, et al. Pathology of *Plasmodium chabaudi* infection and mortality in interleukin-10-deficient mice are ameliorated by anti-tumor necrosis factor alpha and exacerbated by anti-transforming growth factor beta antibodies. *Infect Immun*. 2003; 71:4850–4856. [PubMed: 12933825]
45. Omer FM, Riley EM. Transforming growth factor beta production is inversely correlated with severity of murine malaria infection. *J Exp Med*. 1998; 188:39–48. [PubMed: 9653082]

46. Linke A, Kühn R, Müller W, et al. *Plasmodium chabaudi chabaudi*: Differential susceptibility of gene-targeted mice deficient in IL-10 to an erythrocytic-stage infection. *Exp Parasitol*. 1996; 84:253–263. [PubMed: 8932775]
47. Polanczyk MJ, Carson BD, Subramanian S, et al. Cutting edge: Estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. *J Immunol*. 2004; 173:2227–2230. [PubMed: 15294932]
48. Grimaldi CM, Hill L, Xu X, et al. Hormonal modulation of B cell development and repertoire selection. *Mol Immunol*. 2005; 42:811–820. [PubMed: 15829269]

Figure 1. Mean ($\pm$ SEM) changes from baseline (Day 0) in (A) body mass; (B) rectal temperature; and (C) percentage of RBC; and (D) mean ($\pm$ SEM) percent of parasitemia (parasitized RBC/total RBC) in OVX female mice implanted with either 0.1 mg E₂, 10 mg P₄, 0.1 mg E₂ + 10 mg P₄, or placebo pellets (n = 10/treatment group; N = 40). Data were collected 0, 3, 5, 7, 10, 14, and 21 days after inoculation with *P. chabaudi* parasites. RBC = red blood cells; OVX = ovariectomized; E₂ = 17 β -estradiol; P₄ = progesterone. * P < 0.05 for E₂ and E₂ + P₄-treated females versus P₄- or placebo-treated females, determined by mixed analysis of variance.

Figure 2. Mean ($\pm$ SEM) fold change in (A) interferon-gamma (IFN- γ) and (B) interleukin-10 (IL-10) production by splenocytes, relative to baseline concentrations (Day 0) in OVX female mice implanted with 0.1 mg E₂, 10 mg P₄, 0.1 mg E₂ + 10 mg P₄, or placebo pellets (n = 10/treatment group/time point; N = 160). Data were collected 0, 3, 7, and 21 days after inoculation with *P. chabaudi* parasites. OVX = ovariectomized; E₂ = 17 β -estradiol; P₄ = progesterone. * $P < 0.05$ for E₂ + P₄-treated females versus E₂, P₄-, or placebo-treated

females. $\dagger P < 0.05$ for E₂-treated females versus E₂ + P₄-, P₄-, or placebo-treated females. *P* values determined by 2-way analysis of variance.

Figure 3. Mean (±SEM) anti-*P. chabaudi* (A) immunoglobulin G (IgG) (B) IgG2c, (C) and IgG1 responses, and (D) the ratio of anti-*P. chabaudi* IgG1:IgG2c in OVX female mice implanted with 0.1 mg E₂, 10 mg P₄, 0.1 mg E₂ + 10 mg P₄, or placebo pellets (n = 10/treatment group; N = 40). Serum samples were collected from females 21 days after inoculation with *P. chabaudi* parasites. OVX = ovariectomized; E₂ = 17β-estradiol; P₄ = progesterone. *P < 0.05 for E₂- and E₂ + P₄-treated females versus either P₄- or placebo-treated females, determined by 2-way analysis of variance.