THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 20, Issue of May 17, pp. 17830–17835, 2002
Printed in U.S.A.

Loss of the Peroxisome Proliferation-activated Receptor gamma (PPARγ) Does Not Affect Mammary Development and Propensity for Tumor Formation but Leads to Reduced Fertility*

Received for publication, January 8, 2002, and in revised form, February 25, 2002
Published, JBC Papers in Press, March 7, 2002, DOI 10.1074/jbc.M200186200

Yongzhi Cui‡§, Keiko Miyoshi‡¶, Estefania Claudio‖, Ulrich K. Siebenlist‖, Frank J. Gonzalez, Jodi Flaws‡‡, Kay-Uwe Wagner§§, and Lothar Hennighausen‡§**

*From the ‡Laboratory of Genetics and Physiology, NIDDK, the ‖Laboratory of Immunoregulation, Immune Activation Section, NIAID, and the **Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, Maryland 20892; the ‡‡Department of Epidemiology and Preventive Medicine, University of Maryland Medical School, Baltimore, Maryland 21201; the §§Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska 68198-6805; and the ¶Department of Biochemistry, School of Dentistry, The University of Tokushima, Kuramoto-cho, Tokushima 770-8504, Japan*

The peroxisome proliferation-activated receptor gamma (PPARγ) is expressed in many cell types including mammary epithelium, ovary, macrophages, and B- and T-cells. PPARγ has an anti-proliferative effect in pre-adipocytes and mammary epithelial cells, and treatment with its ligands reduced the progression of carcinogen-induced mammary tumors in mice. Because PPARγ-null mice die *in utero* it has not been possible to study its role in development and tumorigenesis *in vivo*. To investigate whether PPARγ is required for the establishment and physiology of different cell types, a cell-specific deletion of the gene was carried out in mice using the Cre-loxP recombination system. We deleted the *PPARγ* gene in mammary epithelium using WAP-Cre transgenic mice and in epithelial cells, B- and T-cells, and ovary cells using MMTV-Cre mice. The presence of PPARγ was not required for functional development of the mammary gland during pregnancy and for the establishment of B- and T-cells. In addition, no increase in mammary tumors was observed. However, loss of the *PPARγ* gene in oocytes and granulosa cells resulted in impaired fertility. These mice have normal populations of follicles, they ovulate and develop corpora lutea. Although progesterone levels are decreased and implantation rates are reduced, the exact cause of the impaired fertility remains to be determined.

The peroxisome proliferation-activated receptor gamma (PPARγ)[1] is a member of the nuclear receptor superfamily. It is expressed in many cell types, including adipocytes, epithelial cells, B- and T-cells, macrophages, endothelial cells, neutrophils, and smooth muscle cells (1–3). PPARγ regulates gene expression by binding as a heterodimer with retinoid X receptors (RXRs) to specific response elements (PPREs) in the promoter regions of target genes (4, 5). PPARγ ligands mediate a diversity of cellular effects, such as the regulation of adipocytes differentiation, lipid metabolism, and glucose homeostasis (6–8). A versatile array of ligands for PPARγ includes naturally occurring compounds such as fatty acids and the prostaglandin D2 metabolite 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) (9). They also include synthetic compounds such as the thiazolidinedione (TZD) class of insulin-sensitizing agents that are used to treat type II diabetes (10). The extensive use of agonists *in vitro* has resulted in some understanding of PPARγ function in adipogenesis. However, the function of PPARγ is not restricted to adipogenesis and insulin sensitization (11, 12). In peripheral monocytes and macrophages, PPARγ agonists are reported to inhibit the production of inflammatory cytokines (13) and to stimulate lipid metabolism and transport (11). Furthermore PPARγ ligands can induce differentiation and apoptosis in breast (14–17), prostate cancer cells (18), and choriocarcinoma cells (19).

Using a traditional gene-targeting approach, PPARγ-deficient null embryos have been generated, which die at around embryonic day 10 because of defects in placental vascularization that lead to extensive myocardial thinning (20). A single PPARγ-null embryo that was rescued at term exhibited a lethal combination of pathologies, including lipodystrophy and multiple hemorrhages. Because PPARγ is found in a broad spectrum of cell types, tissue-specific gene targeting of the *PPARγ* gene is necessary to expand our knowledge of its physiological role. Conditional disruption of the *PPARγ* gene in macrophages resulted in lowered expression of ABCA1, ABCG1, and apoE and reduced cholesterol efflux (21).

In this report, the role of PPARγ was investigated by deletion of the gene in mammary epithelium, ovary, and B- and T-cells using Cre-loxP-mediated recombination. Mice were generated that carried loxP sites in the first and second intron of the *PPARγ* gene (21) and Cre transgenes under control of the whey acidic protein (*WAP*) gene promoter and the mouse mammary tumor virus long terminal repeat (MMTV-LTR) (22, 23). Through the generation of mice that carry two targeted PPARγ alleles and a Cre transgene, we were able to investigate the roles of PPARγ in mammary gland development and tumorigenesis, in the ovary and the hematopoietic system.

MATERIALS AND METHODS

Transgenic Mice—Conditional PPARγ-null mice were previously generated by floxing exon 2 of the *PPARγ* gene (21). These mice were

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence may be addressed: Laboratory of Genetics and Physiology, NIDDK, National Institutes of Health, Bldg. 8, Rm. 107, 8 Center Dr., Bethesda, MD 20892. Tel.: 301-496-2716; Fax: 301-480-7312; E-mail: hennighausen@nih.gov (to L. H.) or Yongzhic@intra.niddk.nih.gov (to Y. C.).

[1] The abbreviations used are: PPARγ, peroxisome proliferation-activated receptor gamma; WAP, whey acidic protein; MMTV, mouse mammary tumor virus; PMSG, pregnant mare serum gonadotropin; hCG, human chorionic gonadotropin; FACS, fluorescence-activated cell sorter; LTR, long terminal repeat; flox, lox-flank.

mated with MMTV-Cre and WAP-Cre transgenic mice (22) and with ROSA26 reporter mice (24). The genotypes of the mice were determined by PCR analysis. Primers for the *PPARγ* gene were F (5′-ctc caa tgt tct caa act tac-3′), R1 (5′-gat gag tca tgt aag ttg acc-3′), and R2 (5′-gta ttc tat ggc ttc cag tgc-3′), which yielded a 225-bp band from the wild type allele, a 275-bp band from the floxed allele, or a 400-bp band from the null allele (95 °C, 30 s; 60 °C, 30 s; 72 °C, 90 s; 35 cycles). Primers for the Cre transgenes were 5′-tag agc tgt gcc agc ctc ttc c-3′ (which binds in the *WAP* gene promoter), 5′-ggt tct gat ctg agc tct gag tg-3′ (which binds in the MMTV-LTR), and 5′-cat cac tcg ttg cat cga ccg g-3′ (which binds in the Cre sequence). The WAP-Cre transgene produced a 240-bp fragment and MMTV-Cre transgene yielded a 280-bp fragment (95 °C, 30 s; 65 °C, 30 s; 72 °C, 1 min; 30 cycles). The ROSA26 transgene produced a 425-bp product with primers 5′-gat ccg cgc tgg cta ccg gc-3′ and 5′-gga tac tga cga aac gcc tgc c-3′ (95 °C, 30 s; 65 °C, 30 s; 72 °C, 1 min; 30 cycles). All products were separated in 2% agarose Tris acetate/EDTA gels. In the study, all the control mice were PPARγ fl/fl littermates.

Northern Blot—Total RNA from mammary gland samples was isolated at different time points by TRIzol reagent (Invitrogen). Northern blots were prepared with 20 μg of total RNA per lane. The hybridization probe was an approximate 1-kb *Bam*HI/*Spe*I fragment from the 3′-part of the PPARγ cDNA. The identity of the probe was confirmed by sequencing.

Histological Evaluation of Mammary Glands and Ovaries—The inguinal mammary gland was biopsied at the indicated times of development and spread on a glass slide. After fixation for 4 h in Carnoy's solution, the glands were hydrated and stained with carminalum and dehydrated and mounted as described by Kordon *et al.* (25). The glands were photographed, paraffin-embedded, and sectioned at 5 μm. Sections were stained with hematoxylin and eosin. For the β-galactosidase assay, the tissues were fixed in 2% paraformaldehyde, 0.25% glutaraldehyde, and 0.01% Nonidet P-40 in phosphate-buffered saline for 2 h and prestained in 2 mM $MgCl_2$, 0.01% (w/v) sodium deoxycholate, and 0.02% (v/v) Nonidet P-40 in phosphate-buffered saline. Following the prestain, the samples were stained for 24–48 h at 30 °C in 30 mM $K_4Fe(CN)_6$, 30 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$, 0.01% (w/v) sodium deoxycholate, and 0.02% (v/v) Nonidet P-40 in phosphate-buffered saline with 1 mg/ml X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Samples were washed in phosphate-buffered saline and then dehydrated, paraffin-embedded, and sectioned. The sections were counterstained with nuclear fast red.

Ovaries were fixed in Bouin's solution overnight and then washed in 70% ethanol. Paraplast (VWR Scientific, Buffalo Grove, IL)-embedded ovaries were serial-sectioned (8 μm) through the entire tissue, mounted on glass slides, and stained with Weigert's hematoxylin/picric acid methylene blue. Every 10th section was analyzed for the number of primordial, primary, and pre-antral/antral follicle numbers. The number of follicles in every 10th section was multiplied by 8 in order to give an estimate of the total follicle numbers. Only the follicles with a visible nucleus in the oocyte were counted to avoid double counting.

Mammary Epithelium Transplantation—The endogenous epithelium of athymic nude 3-week-old mice was removed as described by DeOme *et al.* (26). A piece of mammary tissue from a mature virgin donor was implanted into the center of the remaining fat pad. Mammary tissues from PPARγ fl/fl; MC(F), and fl/fl mice were transplanted into the right and left sides of the same nude mouse, respectively. To obtain transplanted tissues at term, the hosts were mated 8 weeks after they received the transplant, and the mammary glands were harvested the morning after delivery.

Hormone Level—Progesterone levels were measure by radioimmunoassay using Coat-A-Count (Diagnostic Products Corporations, Los Angeles, CA). Mice were anesthetized, and blood was collected by phlebotomy from the retro-orbital plexus. Serum was separated from cells by centrifugation for 5 min at 5000 rpm. Three 8-week-old PPARγ fl/fl; MC(F) virgin mice and four control mice were checked for estrus cycles, and blood was collected at the estrus day. Serum from six 8-week-old fl/fl; MC(F) and six fl/fl pseudopregnant mice (intraperitoneal injection of 5 IU of pregnant mare serum gonadotropin (PMSG) followed 48 h later by intraperitoneal injection of 5 IU of human chorionic gonadotropin (hCG) every 24 h for a total of 72 h) was analyzed.

Embryo Implantation Sites—Three PPARγ fl/fl; MC(F) adult females and two control females were mated with males of the same strain. The morning of finding a vaginal plug was designated day 0.5 of pregnancy. On day 6.5 of pregnancy, implantation sites were visualized by staining the uterus with 1% ammonium sulfide (Sigma) for 20 min. The implantation sites were identified by the unstained bands along the uterus (the uterus was stained blue).

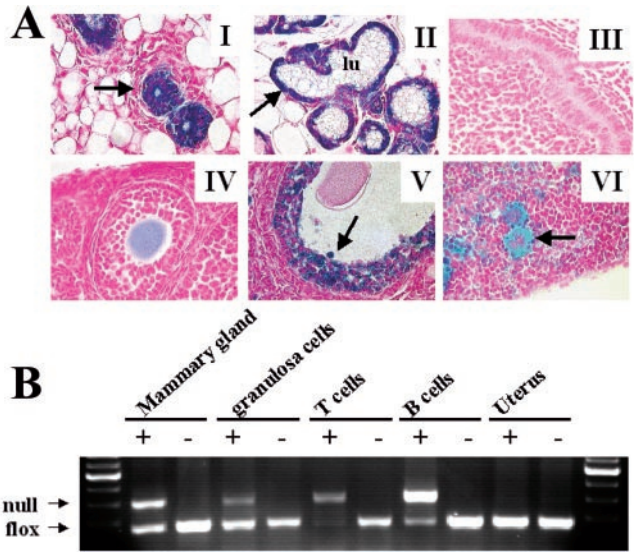

FIG. 1. **Conditional deletion of the *PPARγ* gene in mouse tissue.** *A*, LacZ staining of different tissues in MC(F); Rosa26 mouse: virgin mammary gland (*panel I*); lactation mammary gland (*panel II*), *arrows* point to mammary epithelial cells in *panels I* and *II*, *lu*, lumen; uterus (*panel III*); oocyte (*panel IV*); granulosa cells in ovary, stained blue (*arrow*, *panel V); spleen arrow* points to the megacaryocyte *panel VI. B*, MMTV-Cre(F)-mediated recombination of the *PPARγ* gene in various cell types by PCR analysis. All the samples were from PPARγ fl/fl mice. +, MC(F) positive; −, MC(F) negative. Null band (400 bp) is the recombination product after deletion of exon 2 of the *PPARγ* gene (primers F/R2). The flox band (275 bp) is from the primers F/R1 with one loxP insertion.

Flow Cytometry—Single cell suspensions from spleen were depleted of erythrocytes and 10^6 cells were incubated with different combinations of antibodies for two-color fluorescence surface staining. Data were collected in a FACS calibur flow cytometer (BD PharMingen) and analyzed using CELLQuest software (BD PharMingen). The following monoclonal antibodies were used: anti-B220 (clone RA3-6B2), anti-CD11b (clone MacI), anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7) All the antibodies were purchase from BD PharMingen.

RESULTS

Conditional Deletion of the PPARγ Gene in Mouse Tissues—Because PPARγ-null mice die by day E10 (20), it has not been possible to investigate the function of PPARγ in tissue development and physiology. To overcome this obstacle, we generated mice in which the *PPARγ* gene can be deleted in specific cell types using the Cre-loxP recombination system. Exon 2 of the *PPARγ* gene was flanked by loxP sites to generate PPARγ-floxed mice (21), which were bred with transgenic mice that carry the *Cre* gene under control of either the MMTV-LTR or the *WAP* gene promoter (22, 23). Loss of exon 2 leads to a premature termination of translation (21). Mice that carry floxed PPARγ alleles and the MMTV-Cre transgene are referred to as fl/fl; MC mice and those carrying the WAP-Cre transgene as fl/fl; WC mice. The WAP-Cre transgene is expressed almost exclusively in mammary epithelial cells during pregnancy and lactation, whereas the MMTV-Cre transgene is active in many tissues (23). We used two lines of transgenic mice expressing the MMTV-Cre transgene. While in the D line (MC(D)) the transgene is expressed in several secretory organs and the hematopoietic system (23), it is also expressed in ovarian tissue in the F line (MC(F)). The cell-specificity of Cre expression in the MC(F) line was established using the Rosa26 reporter strain (Fig. 1*A*). Cre activity was found in mammary ductal and alveolar epithelium, in the salivary gland, oocytes, granulosa cells, megacaryocytes, and B- and T-cells, but not in the uterus. We further evaluated the extent of MC(F)-mediated excision of exon 2 of the *PPARγ* gene and its tissue distribution

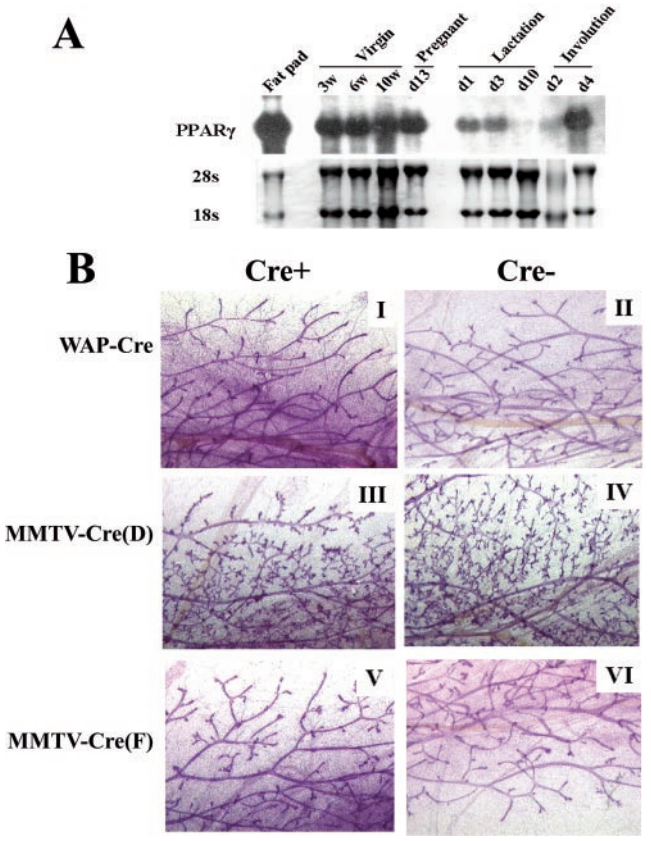

FIG. 2. **The role of PPARγ during mammary development.** *A*, expression pattern of the *PPARγ* gene in mammary gland by Northern blot analysis. *B*, whole mount analyses of mammary tissues from PPARγ fl/fl; Cre and PPARγ fl/fl virgin mice. WAP-Cre and MMTV-Cre(F) mammary tissues were from 8-week-old virgins; MMTV-Cre(D) were from 20-week-old mice.

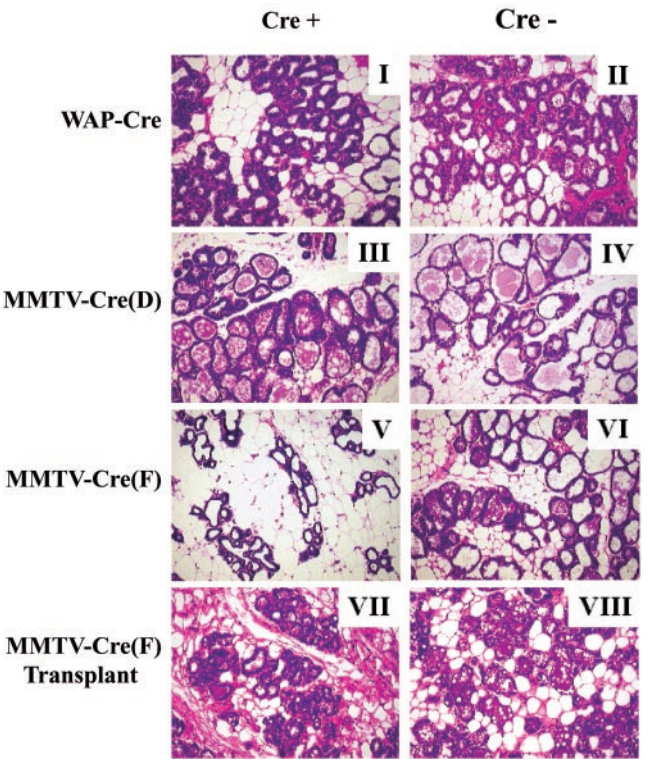

FIG. 3. **Histological analyses of mammary tissues from PPARγ fl/fl; Cre and PPARγ fl/fl mice.** Mammary tissues from PPARγ fl/fl; WC/MC(D)/MC(F) (*panels I, III,* and *V*) and PPARγfl/fl control (*panels II, IV,* and *VI*) mice were harvested at day 1 lactation. *Panels VII* and *VIII,* mammary tissues were harvested from transplanted PPARγ fl/fl; MC(F) mammary epithelium into wild type mice at day 1 lactation. Original magnification: ×200.

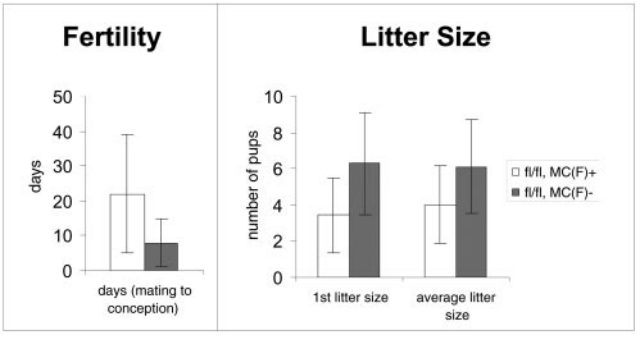

FIG. 4. **Reduced fertility in PPARγ fl/fl; MC(F) mice.** *Left*, average days of mating for PPARγ fl/fl; MC(F) mice conception ($p < 0.01$). The data were from 24 fertile PPARγ fl/fl; MC(F) mice and 32 control mice. *Right*, the average number of pups from the first pregnancy (24 litters from PPARγ fl/fl; MC(F) mice and 32 litters from PPARγ fl/fl control mice) and all pregnancies (46 litters from PPARγ fl/fl; MC(F) mice and 82 litters from PPARγ fl/fl control mice). Data are expressed as mean ± S.D.

by PCR analysis of fl/fl; MC(F) mice. Extensive excision was observed in mammary tissue, B- and T-cells, and to a lesser extent in granulosa cells (Fig. 1*B*). Isolated B- and T-cells from the spleen of the conditional knockout mice exhibited a high recombination efficiency of the *PPARγ* gene. There was no excision of the *PPARγ* gene in the uterus, which demonstrated the absence of Cre recombinase expression.

PPARγ Is Not Required for Functional Mammary Gland Development—It has been shown that the *PPARγ* gene is expressed in both normal mammary epithelial cells and stromal cells (15). We further established the profile of PPARγ during mammary development by using northern blot analyses (Fig. 2*A*). PPARγ mRNA levels were high in virgin tissue and during pregnancy, decreased during lactation, and were reestablished at day 4 of involution. Highest levels of PPARγ mRNA were detected in cleared fat pad, which demonstrates that PPARγ is more abundant in stromal cells than in epithelial cells.

To investigate the role of PPARγ in mammopoiesis, we monitored ductal and alveolar development as well as mammary function in fl/fl; MC and fl/fl; WC mice. Ductal elongation and branching during puberty were normal upon inactivation with both the fl/fl; MC(D) and (F) line (Fig. 2*B*). Similarly, the formation and differentiation of the alveolar compartment appeared normal in fl/fl; WC and fl/fl; MC(D) mice (Fig. 3), and the dams could support their litters. However, pregnancy-mediated alveolar development in fl/fl; MC(F) mice was impaired, and the fat pad was rarely filled with lobules (Fig. 3). Those dams that had only a sparsely developed lobular compartment could not nurse their pups.

To investigate whether the mammary gland phenotype in fl/fl; MC(F) mice was autonomous to the epithelium or caused by systemic defects, we performed mammary epithelial transplants. Epithelium from fl/fl; MC(F) mice was transplanted into the cleared fat pad of athymic nude mice, and mammary development was evaluated at parturition. At parturition, fl/fl; MC(F) mammary epithelium had developed normally, and the fat pad was filled with secretory alveoli (Fig. 3). These results demonstrate that the incomplete mammary development in fl/fl; MC(F) mice was not the result of a primary defect in the epithelium, but rather a secondary defect.

PPARγ Ablation from the Epithelial Compartment Did Not Induce Mammary Tumors—PPARγ is highly expressed in

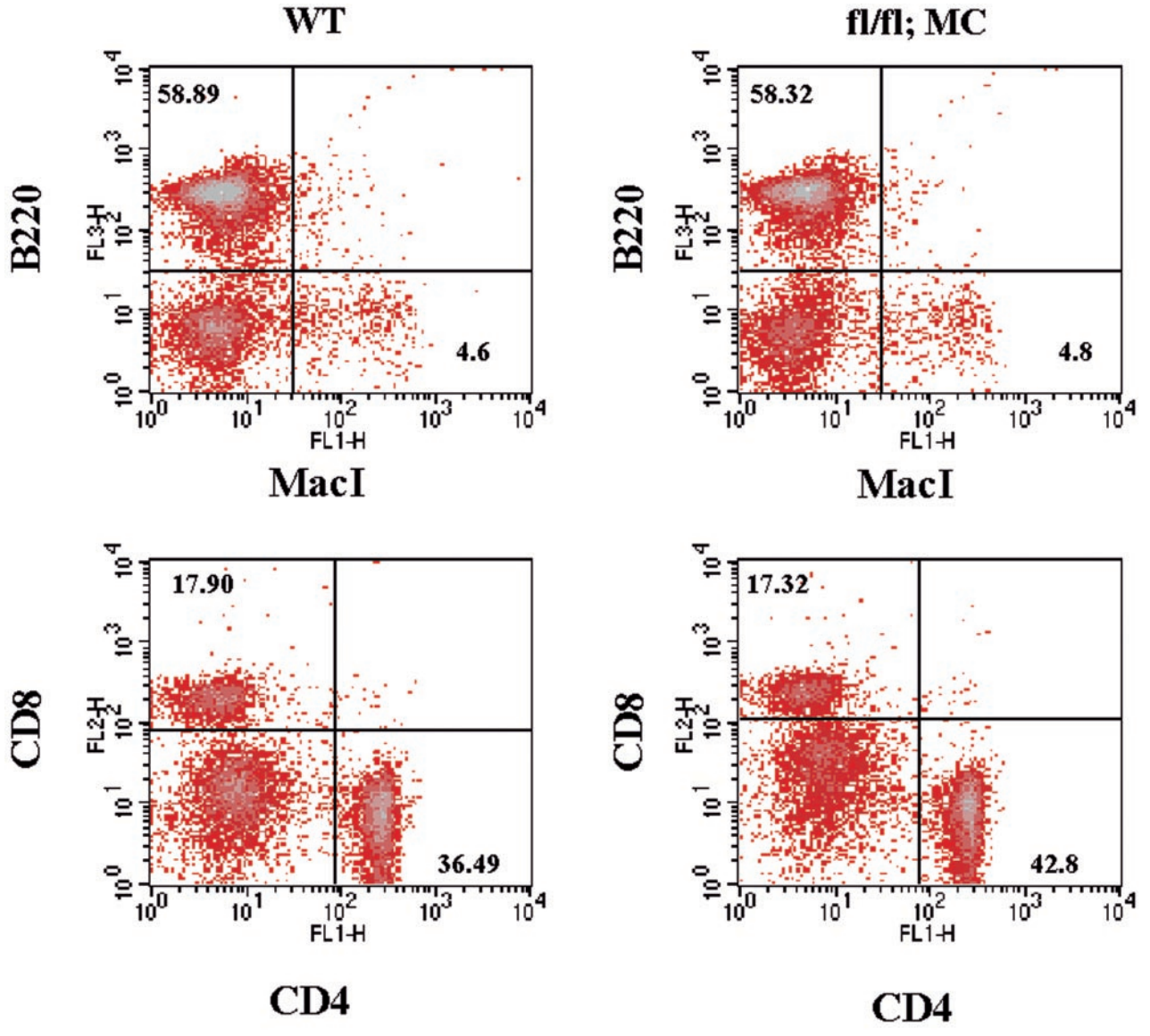

FIG. 5. **Normal B- and T-cell population in spleen of PPARγ fl/fl; MMTV-Cre and control mice.** Two-color FACS analysis of spleen from 6–8-week-old mice. *Upper panels* show staining with anti-B220 and anti-MacI antibodies (B-cell and monocyte surface marker, respectively) and *lower panels* show anti-CD8/anti-CD4 staining (T-cell marker).

breast cancer cell lines and infiltrating ductal breast adenocarcinomas (15), and the activation of PPARγ has been known to inhibit growth and induce apoptosis and terminal differentiation of breast cancer cells *in vitro* and *in vivo* (15, 17). Based on these observations, we hypothesized that the loss of PPARγ would sensitize mice to tumor formation. To investigate this possibility, we observed more than 30 PPARγ fl/fl; MC mice, 20 fl/fl; WC mice, and an equal number of control mice over a period of 12 months. These mice were bred *ad libidum*. None of these mice developed tumors over the 12 months. After 15 months 2 fl/fl; MC mouse and 1 control mouse developed breast tumors. These results suggest PPARγ is not a strong and dominant tumor suppressor.

Loss of PPARγ in the Ovary Results in Reduced Fertility—PPARγ has been found in bovine (27–29), rat (30, 31), and human (32) ovaries. Reverse transcription-PCR results confirmed that the *PPARγ* gene is also expressed in the mouse ovary (data not shown). One-third (11/35) of fl/fl; MC(F) females were infertile, and the remainder exhibited impaired fertility. On the average fl/fl; MC(F) conceived after 22 days of mating, while it took control mice only 8 days ($p < 0.01$) (Fig. 4). In addition, litter sizes of fl/fl; MC(F) dams were small (3 ± 2 pups), while there were 6 ± 3 pups in the fl/fl mice ($p < 0.01$) (Fig. 4).

To investigate the cause of the impaired fertility, we performed morphometric analyses on ovaries from 3-month-old fl/fl; MC(F) ($n = 4$) and fl/fl females ($n = 4$). There was no significant difference in the numbers of primordial (9720 ± 3595 *versus* 9460 ± 5008 in fl/fl; MC(F) *versus* fl/fl ovaries, $p = 0.935$), primary (2940 ± 1253 *versus* 2760 ± 847, $p = 0.819$) and preantral/antral (5240 ± 1201 *versus* 6620 ± 2145, $p = 0.304$) follicles. We also treated fl/fl; MC(F) ($n = 2$) and control ($n = 2$) mice with PMSG (5 IU/mouse) and hCG (5 IU/mouse) to induce superovulation. There was no difference in the number of oocytes (25 *versus* 20) released in response to PMSG.

Reduced fertility of PPARγ fl/fl; MC(F) mice could also be the result of decreased levels of progesterone. We therefore measured progesterone levels in virgin mice at the estrus day ($n = 4$ in each group). Although the progesterone level in fl/fl; MC(F) mice (6.3 ± 3.1 ng/ml) was lower than that in the control (12.5 ± 7.5 ng/ml) mice, the difference was not significant ($p = 0.204$). We also measured the progesterone levels in mice in-

jected with 5 IU of PMSG followed 48 h later by 5 IU of hCG injection. Progesterone levels were 43.6 ± 21.2 ng/ml in fl/fl; MC(F) mice and 51.5 ± 19.6 in fl/fl mice ($n = 6$ in each group). There was no significant difference. We collected and fixed the ovaries from these virgin and pseudopregnant mice, and measured the size of the corpus luteum. There were no differences in morphology or size of the corpus luteum between the fl/fl; MC(F) and control mice ovaries.

We also examined the number of implantations *in utero* of PPARγ fl/fl; MC(F) and control mice. Implantation occurs between days 3.5 and 4 (33), and we counted implantation sites at day 6.5 postcoitus. We found six implantation sites in one of the three PPARγ fl/fl; MC(F) mice but none in the other two. In the two control mice we found five and seven implantation sites, respectively. As described earlier (Fig. 1), Cre was not expressed in the uterus, and the *PPARγ* gene had remained intact.

B- and T-cells Develop in the Absence of PPARγ—A possible role for PPARγ in the differentiation of B- and T-cells has been reported (3, 34). To address whether the development of B- and T-cells requires the presence of PPARγ, we analyzed B- and T-cell populations from spleen using FACS cytometry (Fig. 5). In control mice, B-cells constitute ~50%, T-cells 35%, and macrophages 5% of the total cells in spleen. The same ratio was observed in spleens from PPARγ fl/fl; MC(F) mice. These results suggest that PPARγ is not required for the generation of B- or T-cells.

DISCUSSION

A variety of functions have been attributed to PPARγ. Activation of PPARγ promotes differentiation and induces apoptosis in a broad range of human malignant cell lines, including breast cancer (15, 17), prostate cancer (18), non-small cell lung cancer (35), and liposarcomas (36). Furthermore activation of PPARγ reduces tumor progression in xenograft models of prostate (18) and colon (37) cancers, and it induces regression or stasis of DMBA (9,10-climethyl-1,2-benzanthracene)-induced tumors (14, 16). In contrast with this, other studies show that activation of PPARγ promotes the development of colon tumors in C57BL/6-APC−/+ mice (38, 39). The use of mice in which the *PPARγ* gene is inactivated should shed light on the role of PPARγ on normal development, physiology, and tumorigenesis. Because traditional PPARγ-null mice are embryonic lethal (20), we have now investigated the role of PPARγ through the deletion of the gene in several cell types using Cre-loxP-mediated recombination. Inactivation of the *PPARγ* gene in mammary epithelium with WAP-Cre or MMTV-Cre (D) mice did not interfere with normal development during pregnancy, and lactation was not impaired. Thus unlike other members of the steroid receptor family (40), PPARγ is not essential for ductal and lobulo-alveolar development. Furthermore, we did not observe an increased incidence of mammary tumors. This suggests that PPARγ by itself is not vital for development and is not a dominant tumor suppressor. It is possible that other members of this family, such as PPARα and PPARβ, compensate for the loss of PPARγ, similar to the pRb family (41). The expression of an active oncogene in mammary epithelium devoid of PPARγ (possibly through a transgene) will eventually establish whether PPARγ has any tumor suppressor function in the breast.

Inactivation of the *PPARγ* gene with an MMTV-Cre(F) transgene resulted in impaired fertility and abrogated mammary development. However, lack of functional mammary development is probably a consequence to the ovarian dysfunction for several reasons. Results of mammary epithelial transplants demonstrated the PPARγ-null epithelium could develop into a differentiated mammary gland. *In situ* hybridization has shown that PPARγ mRNA is present in the ovary and primarily in the granulosa cells of developing follicles, but not in the oocytes (31). Following the luteinizing hormone surge, levels of PPARγ mRNA decline suggesting a role in ovarian function (31). Furthermore, the MMTV-Cre(F) line of transgenic mice expresses Cre in oocytes, granulosa cells, and the corpora lutea, and the impaired fertility could be the result of subfunctional physiology of these cell types.

PPARγ fl/fl; MC(F) mice appeared to ovulate normally but exhibited impaired implantation. Because Cre was not expressed in uterine tissue and the *PPARγ* gene was intact, uterine dysfunction can be ruled out. In the mouse, secretion of progesterone from newly formed corpora lutea, accompanied by preimplantation ovarian estrogen secretion on day 4 of pregnancy, is critical for the establishment of uterine receptivity for implantation (42). The activation of PPARγ has been shown to affect progesterone production. PPARγ ligands inhibited progesterone production in cultured human and porcine granulosa cells (43); however, they stimulated the secretion of both progesterone and E2 in cultured rat granulosa cells (31). In our *in vivo* study, progesterone levels were reduced in virgin mice upon inactivation of the *PPARγ* gene in granulosa cells and the corpora lutea. These mice had normal follicle numbers and normal estrous cycles. When stimulated by PMSG/hCG injection the progesterone levels increased to the normal range, and the morphology and size of the corpora lutea were normal, suggesting the ovary was functional upon exogenous hormone challenging. Under physiological conditions, the ovarian function might not be sufficient to induce implantation, which could explain the reduced fertility of PPARγ fl/fl; MC(F) mice.

REFERENCES

1. Spiegelman, B. M. (1997) *Eur. J. Med. Res.* **2,** 457–464
2. Law, R. E., Goetze, S., Xi, X. P., Jackson, S., Kawano, Y., Demer, L., Fishbein, M. C., Meehan, W. P., and Hsueh, W. A. (2000) *Circulation* **101,** 1311–1318
3. Clark, R. B., Bishop-Bailey, D., Estrada-Hernandez, T., Hla, T., Puddington, L., and Padula, S. J. (2000) *J. Immunol.* **164,** 1364–1371
4. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992) *Nature* **358,** 771–774
5. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) *Genes Dev.* **8,** 1224–1234
6. Rosen, E. D., Sarraf, P., Troy, A. E., Bradwin, G., Moore, K., Milstone, D. S., Spiegelman, B. M., and Mortensen, R. M. (1999) *Mol. Cell* **4,** 611–617
7. Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsubamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y., Tanaka, S., Shiota, K., Kitamura, T., Fujita, T., Ezaki, O., Aizawa, S., Nagai, R., Tobe, K., Kimura, S., and Kadowaki, T. (1999) *Mol Cell* **4,** 597–609
8. Chawla, A., Schwarz, E. J., Dimaculangan, D. D., and Lazar, M. A. (1994) *Endocrinology* **135,** 798–800
9. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) *Cell* **83,** 803–812
10. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliewer, S. A. (1995) *J. Biol. Chem.* **270,** 12953–12956
11. Spiegelman, B. M., and Flier, J. S. (1996) *Cell* **87,** 377–389
12. Tontonoz, P., Nagy, L., Alvarez, J. G., Thomazy, V. A., and Evans, R. M. (1998) *Cell* **93,** 241–252
13. Spiegelman, B. M. (1998) *Cell* **93,** 153–155
14. Pighetti, G. M., Novosad, W., Nicholson, C., Hitt, D. C., Hansens, C., Hollingsworth, A. B., Lerner, M. L., Brackett, D., Lightfoot, S. A., and Gimble, J. M. (2001) *Anticancer Res.* **21,** 825–829
15. Mueller, E., Sarraf, P., Tontonoz, P., Evans, R. M., Martin, K. J., Zhang, M., Fletcher, C., Singer, S., and Spiegelman, B. M. (1998) *Mol. Cell* **1,** 465–470
16. Mehta, R. G., Williamson, E., Patel, M. K., and Koeffler, H. P. (2000) *J. Natl. Cancer Inst.* **92,** 418–423
17. Elstner, E., Muller, C., Koshizuka, K., Williamson, E. A., Park, D., Asou, H., Shintaku, P., Said, J. W., Heber, D., and Koeffler, H. P. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 8806–8811
18. Kubota, T., Koshizuka, K., Williamson, E. A., Asou, H., Said, J. W., Holden, S., Miyoshi, I., and Koeffler, H. P. (1998) *Cancer Res.* **58,** 3344–3352
19. Keelan, J. A., Sato, T. A., Marvin, K. W., Lander, J., Gilmour, R. S., and Mitchell, M. D. (1999) *Biochem. Biophys. Res. Commun.* **262,** 579–585
20. Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A., and Evans, R. M. (1999) *Mol. Cell* **4,** 585–595
21. Akiyama, T. E., S. S., Lambert, G., Nicol, C., Matsusue, K., Pimprale, S., Lee, Y., Ricote, M., Glass, C., Brewer, Jr H. B., Gonzalez, F. (2002) *Mol. Cell. Biol.* **22,** 2607–2619
22. Wagner, K. U., Wall, R. J., St-Onge, L., Gruss, P., Wynshaw-Boris, A., Garrett, L., Li, M., Furth, P. A., and Hennighausen, L. (1997) *Nucleic Acids Res.* **25,** 4323–4330
23. Wagner, K. U., McAllister, K., Ward, T., Davis, B., Wiseman, R., and

Hennighausen, L. (2001) *Transgenic Res.* **10,** 545–553
24. Soriano, P. (1999) *Nat. Genet.* **21,** 70–71
25. Kordon, E. C., McKnight, R. A., Jhappan, C., Hennighausen, L., Merlino, G., and Smith, G. H. (1995) *Dev. Biol.* **168,** 47–61
26. DeOme, K. B., Faulkin, L. J., Jr., Bern, H. A., and Blair, P. E. (1959) *Cancer Res.* **19,** 515–520
27. Sundvold, H., Brzozowska, A., and Lien, S. (1997) *Biochem. Biophys. Res. Commun.* **239,** 857–861
28. Lohrke, B., Viergutz, T., Shahi, S. K., Pohland, R., Wollenhaupt, K., Goldammer, T., Walzel, H., and Kanitz, W. (1998) *J. Endocrinol.* **159,** 429–439
29. Viergutz, T., Loehrke, B., Poehland, R., Becker, F., and Kanitz, W. (2000) *J. Reprod. Fertil.* **118,** 153–161
30. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) *Endocrinology* **137,** 354–366
31. Komar, C. M., Braissant, O., Wahli, W., and Curry, T. E., Jr. (2001) *Endocrinology* **142,** 4831–4838
32. Lambe, K. G., and Tugwood, J. D. (1996) *Eur. J. Biochem.* **239,** 1–7
33. Binart, N., Helloco, C., Ormandy, C. J., Barra, J., Clement-Lacroix, P., Baran, N., and Kelly, P. A. (2000) *Endocrinology* **141,** 2691–2697
34. Padilla, J., Kaur, K., Cao, H. J., Smith, T. J., and Phipps, R. P. (2000) *J. Immunol.* **165,** 6941–6948
35. Chang, T. H., and Szabo, E. (2000) *Cancer Res.* **60,** 1129–1138
36. Tontonoz, P., Singer, S., Forman, B. M., Sarraf, P., Fletcher, J. A., Fletcher, C. D., Brun, R. P., Mueller, E., Altiok, S., Oppenheim, H., Evans, R. M., and Spiegelman, B. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 237–241
37. Sarraf, P., Mueller, E., Jones, D., King, F. J., DeAngelo, D. J., Partridge, J. B., Holden, S. A., Chen, L. B., Singer, S., Fletcher, C., and Spiegelman, B. M. (1998) *Nat. Med.* **4,** 1046–1052
38. Lefebvre, A. M., Chen, I., Desreumaux, P., Najib, J., Fruchart, J. C., Geboes, K., Briggs, M., Heyman, R., and Auwerx, J. (1998) *Nat. Med.* **4,** 1053–1057
39. Saez, E., Tontonoz, P., Nelson, M. C., Alvarez, J. G., Ming, U. T., Baird, S. M., Thomazy, V. A., and Evans, R. M. (1998) *Nat. Med.* **4,** 1058–1061
40. Hennighausen, L., and Robinson, G. W. (2001) *Dev. Cell.* **1,** 467–475
41. Robinson, G. W., Wagner, K. U., and Hennighausen, L. (2001) *Oncogene* **20,** 7115–7119
42. Reese, J., Binart, N., Brown, N., Ma, W. G., Paria, B. C., Das, S. K., Kelly, P. A., and Dey, S. K. (2000) *Endocrinology* **141,** 1872–1881
43. Gasic, S., Bodenburg, Y., Nagamani, M., Green, A., and Urban, R. J. (1998) *Endocrinology* **139,** 4962–4966