

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

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The peroxisome proliferation-activated receptor gamma (PPAR $\gamma$ ) is expressed in many cell types including mammary epithelium, ovary, macrophages, and B- and T-cells. PPAR $\gamma$  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 PPAR $\gamma$ -null mice die *in utero* it has not been possible to study its role in development and tumorigenesis *in vivo*. To investigate whether PPAR $\gamma$  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 PPAR $\gamma$  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 PPAR $\gamma$  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 PPAR $\gamma$  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 (PPAR $\gamma$ )<sup>1</sup> 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). PPAR $\gamma$  regulates gene expression by binding as a heterodimer with retinoid X recep-

tors (RXRs) to specific response elements (PPREs) in the promoter regions of target genes (4, 5). PPAR $\gamma$  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 PPAR $\gamma$  includes naturally occurring compounds such as fatty acids and the prostaglandin D2 metabolite 15-deoxy- $\Delta$ 12,14-prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>) (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 PPAR $\gamma$  function in adipogenesis. However, the function of PPAR $\gamma$  is not restricted to adipogenesis and insulin sensitization (11, 12). In peripheral monocytes and macrophages, PPAR $\gamma$  agonists are reported to inhibit the production of inflammatory cytokines (13) and to stimulate lipid metabolism and transport (11). Furthermore PPAR $\gamma$  ligands can induce differentiation and apoptosis in breast (14–17), prostate cancer cells (18), and choriocarcinoma cells (19).

Using a traditional gene-targeting approach, PPAR $\gamma$ -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 PPAR $\gamma$ -null embryo that was rescued at term exhibited a lethal combination of pathologies, including lipodystrophy and multiple hemorrhages. Because PPAR $\gamma$  is found in a broad spectrum of cell types, tissue-specific gene targeting of the PPAR $\gamma$  gene is necessary to expand our knowledge of its physiological role. Conditional disruption of the PPAR $\gamma$  gene in macrophages resulted in lowered expression of ABCA1, ABCG1, and apoE and reduced cholesterol efflux (21).

In this report, the role of PPAR $\gamma$  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 PPAR $\gamma$  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 PPAR $\gamma$  alleles and a Cre transgene, we were able to investigate the roles of PPAR $\gamma$  in mammary gland development and tumorigenesis, in the ovary and the hematopoietic system.

## MATERIALS AND METHODS

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

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<sup>1</sup> The abbreviations used are: PPAR $\gamma$ , 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 sorting; 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 *PPAR $\gamma$*  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 tgg ttg cat cga cgc 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 cgc cgc tgg cta cgc 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 *PPAR $\gamma$*  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  $\mu$ g of total RNA per lane. The hybridization probe was an approximate 1-kb *Bam*HI/*Spe*I fragment from the 3'-part of the *PPAR $\gamma$*  cDNA. The identity of the probe was confirmed by sequencing.

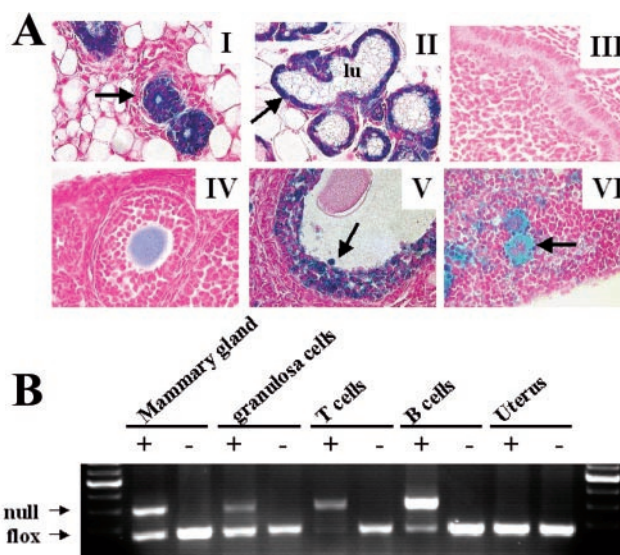
**Histological Evaluation of Mammary Glands and Ovaries**—The inguinal mammary gland was biopsied at the indicated times of development and spread on a glass slide. After fixation for 4 h in Carnoy's solution, the glands were hydrated and stained with carmalum and dehydrated and mounted as described by Kordon *et al.* (25). The glands were photographed, paraffin-embedded, and sectioned at 5  $\mu$ m. Sections were stained with hematoxylin and eosin. For the  $\beta$ -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<sub>2</sub>, 0.01% (w/v) sodium deoxycholate, and 0.02% (v/v) Nonidet P-40 in phosphate-buffered saline. Following the pre-stain, the samples were stained for 24–48 h at 30 °C in 30 mM K<sub>4</sub>Fe(CN)<sub>6</sub>, 30 mM K<sub>3</sub>Fe(CN)<sub>6</sub>, 2 mM MgCl<sub>2</sub>, 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- $\beta$ -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  $\mu$ m) 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 *PPAR $\gamma$*  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 measured by radioimmunoassay using Coat-A-Count (Diagnostic Products Corporation, 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 *PPAR $\gamma$*  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 *PPAR $\gamma$*  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).

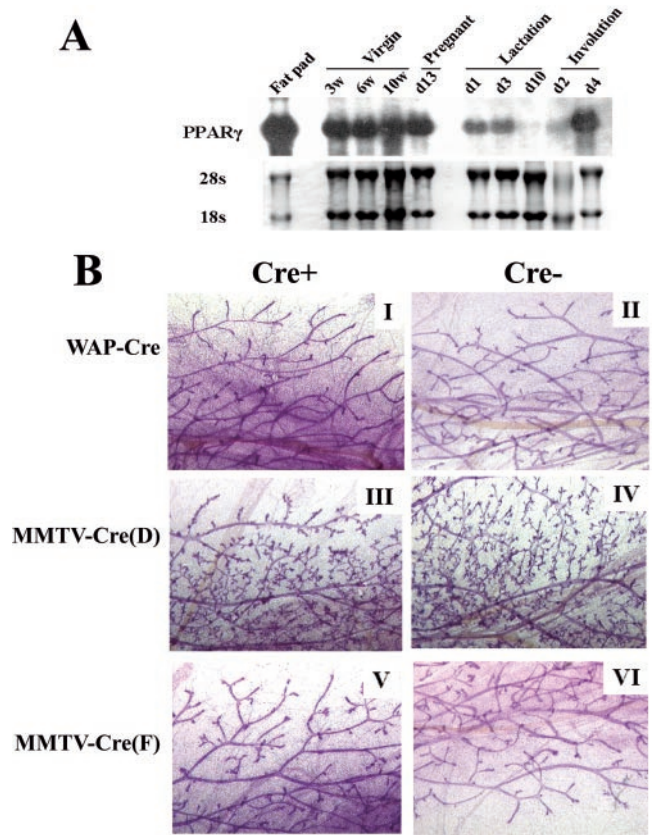


**FIG. 1. Conditional deletion of the *PPAR $\gamma$*  gene in mouse tissue.** A, LacZ staining of different tissues in MC(F); Rosa26 mouse: virgin mammary gland (panel I); lactation mammary gland (panel II), arrows point to mammary epithelial cells in panels I and II, lu, lumen; uterus (panel III); 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 *PPAR $\gamma$*  gene in various cell types by PCR analysis. All the samples were from *PPAR $\gamma$*  fl/fl mice. +, MC(F) positive; -, MC(F) negative. Null band (400 bp) is the recombination product after deletion of exon 2 of the *PPAR $\gamma$*  gene (primers F/R2). The floxed 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<sup>6</sup> cells were incubated with different combinations of antibodies for two-color fluorescence surface staining. Data were collected in a FACS caliber 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 Mac1), anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7). All the antibodies were purchased from BD Pharmingen.

## RESULTS

**Conditional Deletion of the *PPAR $\gamma$*  Gene in Mouse Tissues**—Because *PPAR $\gamma$* -null mice die by day E10 (20), it has not been possible to investigate the function of *PPAR $\gamma$*  in tissue development and physiology. To overcome this obstacle, we generated mice in which the *PPAR $\gamma$*  gene can be deleted in specific cell types using the Cre-loxP recombination system. Exon 2 of the *PPAR $\gamma$*  gene was flanked by loxP sites to generate *PPAR $\gamma$* -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 *PPAR $\gamma$*  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. 1A). 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 *PPAR $\gamma$*  gene and its tissue distribution



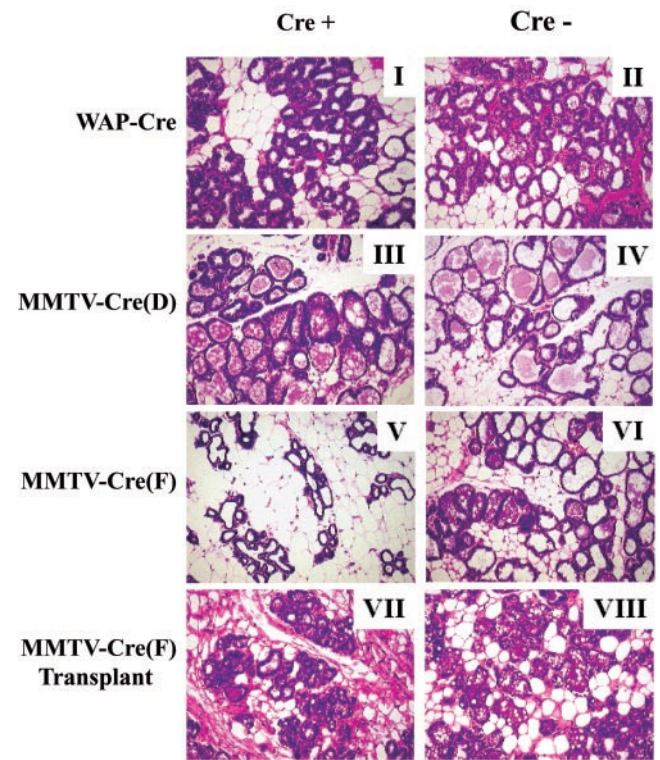
**FIG. 2. The role of PPAR $\gamma$  during mammary development.** *A*, expression pattern of the PPAR $\gamma$  gene in mammary gland by Northern blot analysis. *B*, whole mount analyses of mammary tissues from PPAR $\gamma$  fl/fl; Cre and PPAR $\gamma$  fl/fl virgin mice. WAP-Cre and MMTV-Cre(D) mammary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

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 PPAR $\gamma$  gene. There was no excision of the PPAR $\gamma$  gene in the uterus, which demonstrated the absence of Cre recombinase expression.

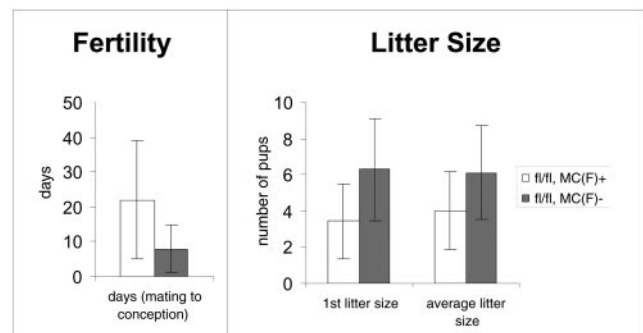
**PPAR $\gamma$  Is Not Required for Functional Mammary Gland Development**—It has been shown that the PPAR $\gamma$  gene is expressed in both normal mammary epithelial cells and stromal cells (15). We further established the profile of PPAR $\gamma$  during mammary development by using northern blot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

To investigate the role of PPAR $\gamma$  in mammapoiesis, 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



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



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

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.

**PPAR $\gamma$  Ablation from the Epithelial Compartment Did Not Induce Mammary Tumors**—PPAR $\gamma$  is highly expressed in

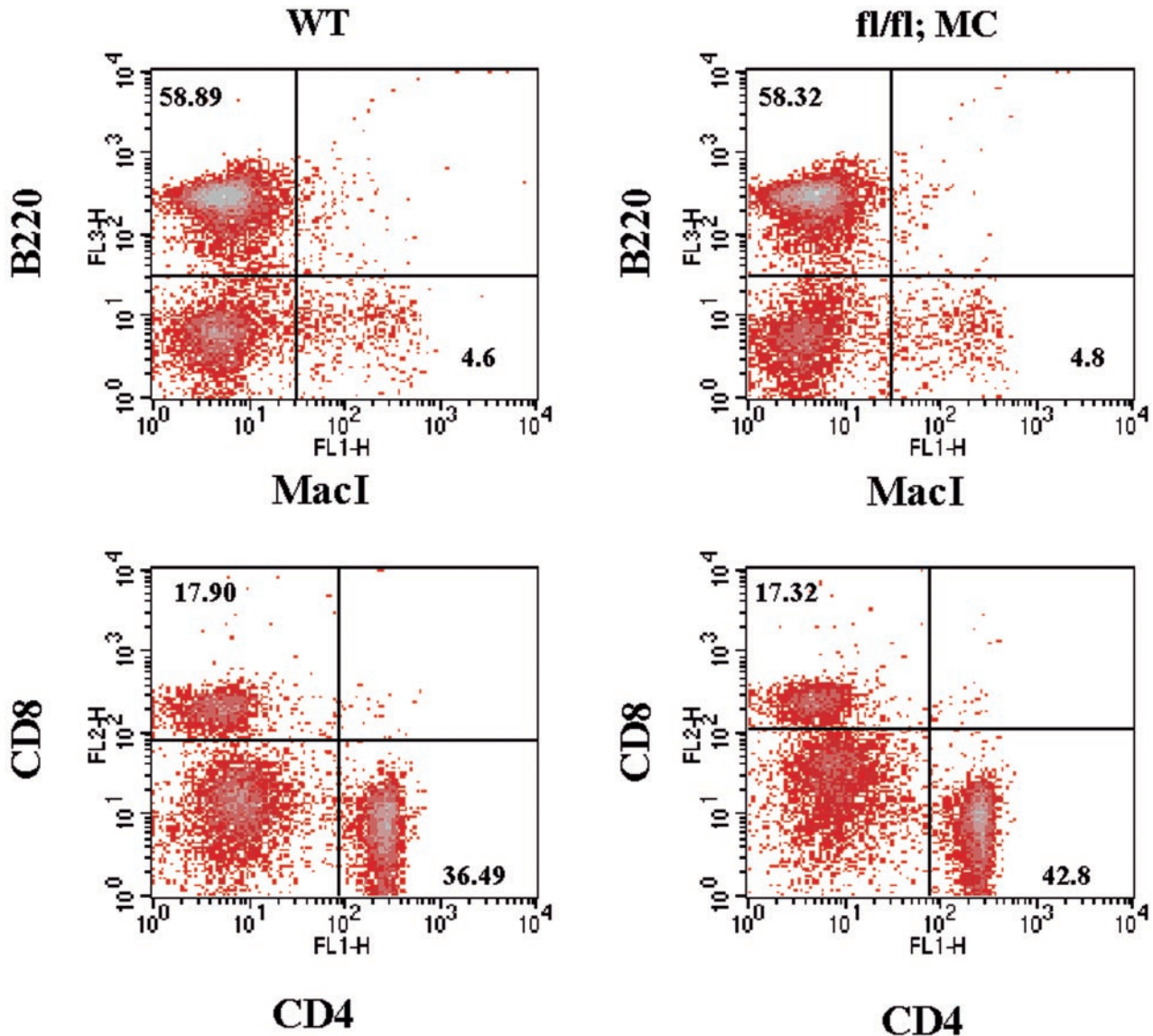


FIG. 5. Normal B- and T-cell population in spleen of PPAR $\gamma$  fl/fl; MMTV-Cre and control mice. Two-color FACS analysis of spleen from 6–8-week-old mice. Upper panels show staining with anti-B220 and anti-MacI antibodies (B-cell and monocyte surface marker, respectively) and lower panels show anti-CD8/anti-CD4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

breast cancer cell lines and infiltrating ductal breast adenocarcinomas (15), and the activation of PPAR $\gamma$  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 PPAR $\gamma$  would sensitize mice to tumor formation. To investigate this possibility, we observed more than 30 PPAR $\gamma$  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 libitum*. 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 PPAR $\gamma$  is not a strong and dominant tumor suppressor.

**Loss of PPAR $\gamma$  in the Ovary Results in Reduced Fertility**—PPAR $\gamma$  has been found in bovine (27–29), rat (30, 31), and human (32) ovaries. Reverse transcription-PCR results confirmed that the PPAR $\gamma$  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 ( $3 \pm 2$  pups), while there were  $6 \pm 3$  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 ( $9720 \pm 3595$  versus  $9460 \pm 5008$  in fl/fl; MC(F) versus fl/fl ovaries,  $p = 0.935$ ), primary ( $2940 \pm 1253$  versus  $2760 \pm 847$ ,  $p = 0.819$ ) and preantral/antral ( $5240 \pm 1201$  versus  $6620 \pm 2145$ ,  $p = 0.304$ ) 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 PPAR $\gamma$  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 ( $6.3 \pm 3.1$  ng/ml) was lower than that in the control ( $12.5 \pm 7.5$  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  $43.6 \pm 21.2$  ng/ml in fl/fl; MC(F) mice and  $51.5 \pm 19.6$  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 PPAR $\gamma$  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 PPAR $\gamma$  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 PPAR $\gamma$  gene had remained intact.

**B- and T-cells Develop in the Absence of PPAR $\gamma$** —A possible role for PPAR $\gamma$  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 PPAR $\gamma$ , 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 PPAR $\gamma$  fl/fl; MC(F) mice. These results suggest that PPAR $\gamma$  is not required for the generation of B- or T-cells.

#### DISCUSSION

A variety of functions have been attributed to PPAR $\gamma$ . Activation of PPAR $\gamma$  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 PPAR $\gamma$  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 PPAR $\gamma$  promotes the development of colon tumors in C57BL/6-APC- $+/+$  mice (38, 39). The use of mice in which the PPAR $\gamma$  gene is inactivated should shed light on the role of PPAR $\gamma$  on normal development, physiology, and tumorigenesis. Because traditional PPAR $\gamma$ -null mice are embryonic lethal (20), we have now investigated the role of PPAR $\gamma$  through the deletion of the gene in several cell types using Cre-loxP-mediated recombination. Inactivation of the PPAR $\gamma$  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), PPAR $\gamma$  is not essential for ductal and lobulo-alveolar development. Furthermore, we did not observe an increased incidence of mammary tumors. This suggests that PPAR $\gamma$  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 PPAR $\alpha$  and PPAR $\beta$ , compensate for the loss of PPAR $\gamma$ , similar to the pRb family (41). The expression of an active oncogene in mammary epithelium devoid of PPAR $\gamma$  (possibly through a transgene) will eventually establish whether PPAR $\gamma$  has any tumor suppressor function in the breast.

Inactivation of the PPAR $\gamma$  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 PPAR $\gamma$ -null epithelium could develop into a differentiated mammary gland. *In situ* hybridization has shown that PPAR $\gamma$  mRNA is present in the ovary and primar-

ily in the granulosa cells of developing follicles, but not in the oocytes (31). Following the luteinizing hormone surge, levels of PPAR $\gamma$  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.

PPAR $\gamma$  fl/fl; MC(F) mice appeared to ovulate normally but exhibited impaired implantation. Because Cre was not expressed in uterine tissue and the PPAR $\gamma$  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 PPAR $\gamma$  has been shown to affect progesterone production. PPAR $\gamma$  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 PPAR $\gamma$  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 PPAR $\gamma$  fl/fl; MC(F) mice.

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