Rescue of Female Infertility from the Loss of Cyclooxygenase-2 by Compensatory Up-regulation of Cyclooxygenase-1 Is a Function of Genetic Makeup*

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Cyclooxygenase-2 (COX-2), an inducible rate-limiting enzyme in prostaglandin biosynthesis, is implicated in various physiological and pathological processes including female fertility, renal function, angiogenesis, inflammation, and tumorigenesis. We showed previously that targeted deletion of Ptgs2 encoding COX-2, but not Ptgs1 encoding COX-1, in C57BL/6J/129 mice produces complete female infertility resulting from multiple reproductive failures spanning ovulation, fertilization, and implantation. Here we show that Ptgs2 null mice on a CD1 background have dramatically improved female fertility including ovulation, fertilization, and implantation, giving rise to live births. We provide evidence that this improved fertility in CD1 Ptgs2 null mice is the result of a compensatory up-regulation of Ptgs1 which does not occur in C57BL/6J/129 mice missing Ptgs2. These results clearly demonstrate for the first time that COX-1 can replace specific functions of COX-2 in vivo in the context of genetic disparity. In light of this finding, the therapeutic use and efficacy of COX-2-specific inhibitors among human populations without regard for genetic and ethnic diversities should be revisited.

Cyclooxygenases, COX-1 and COX-2, mediate the conversion of arachidonic acid into prostaglandin (PG)H₂, which is then converted to various PGs by specific synthases (1). Although both COX-1 and COX-2 isoforms are encoded by separate genes, Ptgs1 and Ptgs2, respectively, they share similar structural and kinetic properties and show distinct cell-specific expression and regulation (1). COX-1 is thought to serve “housekeeping” functions as a constitutive enzyme. COX-2, however, is highly inducible by diverse stimuli including cytokines, growth factors, mitogens, and tumor promoters and regulates inflammation, differentiation, mitogenesis, and angiogenesis (1).

PGs are implicated in various physiological and pathological functions because of their vasoactive, mitogenic, and differentiating properties (2). Ovulation and implantation are considered analogous to “proinflammatory” responses, and thus participation of PGs in these processes has long been speculated. For example, gonadotropin-mediated Ptgs2 expression in ovarian follicles preceding ovulation is consistent with the presumed role of PGs in follicular rupture during ovulation (3, 4). PGs are also implicated as important mediators of increased localized endometrial vascular permeability at the site of the blastocyst during implantation, a prerequisite condition for this process. Distinct expression patterns of COX isoforms in the peri-implantation mouse uterus suggest their roles in uterine biology and implantation (5). The relative importance of COX isoforms in female fertility has been characterized by gene targeting studies in mice. The loss of the Ptgs2 gene leads to multiple reproductive failures that include ovulation, fertilization, implantation, and decidualization (6, 7), whereas Ptgs1-deficient mice have normal fertility except for parturition defects (8, 9).

There is now increasing evidence that mutation of a gene often results in substantially altered phenotypes depending on the genetic background of mice in which the mutation is maintained (10–14). For example, the targeting of the epidermal growth factor receptor (Egfr) locus showed that although epidermal growth factor receptor deficiency on a CF1 background results in peri-implantation death caused by the degeneration of the inner cell mass, this deficiency on a 129/Sv background led to placental defects giving rise to mid-gestational embryonic lethality. In contrast, Egfr mutation on a CD1 background produced early postnatal lethality with abnormal functions of the skin, kidney, brain, liver, and gastrointestinal tract (11). This study was followed by several other studies showing variable phenotypes in mice with different genetic backgrounds for deficiencies in transforming growth factor-β1 (12), leptin (13) and centromere protein B (14). These differential genotype-phenotype effects were suggested to involve modifier genes (10, 12), although experimental identification of the modifier genes remains largely unknown. We now show for the first time that a compensatory up-regulation of Ptgs1 markedly improves female fertility under Ptgs2 deficiency in a particular genetic context. Furthermore, COX-1, which is usually considered as a constitutive enzyme, becomes tightly regulated and functions...
as an inducible isoform similar to the native COX-2 under such a condition. Our present observations of differential Ptgs2-deficient phenotypes on different genetic backgrounds have significant clinical implications, as nonsteroidal anti-inflammatory drugs and COX-2-selective inhibitors are widely used for the treatment of inflammatory diseases without regard for genetic diversities among human populations (15).

EXPERIMENTAL PROCEDURES

Mice—The disruption of the Ptgs2 gene was originally carried out in AB2.1 (129) embryonic stem cells by homologous recombination as described previously (6). PCR of genomic DNA and blood urea nitrogen tests determined the genotypes (6, 7). Because there is evidence that the genetic background of mice contributes to different phenotypes (10–14), to explore whether Ptgs2 null female mice on different genetic backgrounds exhibit different reproductive phenotypes, we introduced Ptgs2 deficiency in CD1 mice by crossing with C57BL/6J/129 Ptgs2+/− mice generated originally. In brief, C57BL/6J/129 Ptgs2+/− females were crossed to CD1 wild-type males producing an F1 generation. F1 Ptgs2+/− females were then back-crossed to CD1 wild-type males, and the process was continued for eight generations. Crossing heterozygous females with heterozygous males of the same genetic background generated littermate wild-type and homozygous Ptgs2 null mice for experiments. The presence of variable genotypes of secretory phospholipase A2-IA (sPLA2-IA), as expected, in CD1 wild-type and mutant mice in contrast to the total absence of sPLA2-IA caused by natural null mutation in C57BL/6J/129 mice provides evidence that this back-crossing protocol restored most of the CD1 genetic elements. All of the mice used were housed in the Institutional Animal Care Facility according to National Institutes of Health and institutional guidelines for laboratory animals.

Ovulation and Fertilization—To examine ovulation and fertilization, mice were mated with fertile males (day 1 = vaginal plug) with the same background and genotypes, respectively. Mice were killed on days 1 and 2 of pregnancy, and oviducts were flushed with Whitten’s medium to recover eggs and embryos. Their morphology was examined under an inverted microscope. To induce superovulation, mice were given an intraperitoneal injection of 5 IU of equine chorionic gonadotrophin (eCG) followed by a second injection of 5 IU of human CG (hCG) 48 h later. Mice were placed overnight with fertile males and checked for vaginal plugs the next morning.

Implantation, Decidualization, Postimplantation Embryonic Growth, and Pregnancy—Implantation sites on days 6–8 of pregnancy were visualized by an intravaginal injection of Chicago blue dye solution (17). Uterine horns on days 5 and 6 without implantation sites were flushed with Whitten’s medium to recover unimplanted blastocysts. To induce experimental decidualization, pseudopregnant mice received an intraluminal infusion of 25 μl of sesame oil in one uterine horn on day 4 of pregnancy, and were killed 4 days later. Uterine weights of the infused and noninfused (control) horns were recorded, and the fold increases in uterine weights were used as an index of decidualization (7). To examine rescue of decidualization by Ptgs1 in Ptgs2 null mice, a COX-1-selective inhibitor SC560 was used. It was prepared in 0.5% (w/v) methylcellulose and 0.1% (v/v) polysorbate 80 in water. First oral gavage (2 mg/kg of body weight) was given at 9 a.m. and 5 p.m. until day 7. Mice were sacrificed on day 8.

To examine postimplantation embryonic growth, day 12 implantation sites and embryos were examined as described previously (18). In brief, dissected day 12 implantation sites were weighed individually, fixed in 10% formalin overnight, and dissected to isolate embryos. Isolated embryos were weighed individually, and their images were captured to examine the size and gross morphology. To examine pregnancy outcome in Ptgs2−/− mice, vaginal plug-positive mice were observed for delivery of pups at term. Litter size and days of pregnancy were recorded.

Blotting—Mating females with wild-type vasectomized males induced pseudopregnancy. Day 4 wild-type blastocysts were transferred into uterine lumens of day 4 pseudopregnant recipients (7, 17). On days 5 and 6, the number of implantation sites was recorded after intravaginal injections of Chicago blue dye solution (17). To examine postimplantation embryo growth, recipients were killed on day 12 of pregnancy.

| Genotype | No. of sperm-positive mice | No. of mice with term pregnancy | Litter size |
|----------|---------------------------|-------------------------------|-------------|
| Wild-type | 10                        | 10 (100%)                     | 14.5 ± 0.8  |
| CD1 Ptgs2−/− | 44                      | 20 (45%)                      | 3.7 ± 0.8  |

Protaglandin Assays—PGs were quantitated using gas chromatography/negative ion chemical ionization mass spectrometric assays as described previously (19).

In Situ Hybridization—Frozen sections were hybridized with 35S-labeled cRNA probes to mouse Ptgs1 and Ptgs2, respectively, as described previously (20).

Western Blotting—The levels of COX-1 and COX-2 in uterine tissues on different days of pregnancy or treatments were analyzed by immunoblotting as described previously (21).

RESULTS

Ovulation and Fertilization Are Improved in CD1 Ptgs2−/− Mice—To explore whether Ptgs2 null female mice on different genetic backgrounds exhibit different reproductive phenotypes, we introduced Ptgs2 deficiency in CD1 mice by crossing with C57BL/6J/129 Ptgs2−/− mutant mice. Improved female fertility with the birth of viable pups in CD1 Ptgs2−/− mice was observed (Table I) compared with complete pregnancy failure in C57BL/6J/129 null mice as observed previously (7). To determine the cause(s) of this improved fertility in CD1 Ptgs2−/− female mice, we examined in detail their reproductive phenotypes during pregnancy.

To examine the ovulation and fertilization status, the number of ovulated eggs and two-cell embryos on day 2 of pregnancy were recorded. To our surprise, a remarkable improvement in ovulation was noted in CD1 Ptgs2−/− mice as opposed to our previous observation of severely compromised ovulation in C57BL/6J/129 mutant mice (7, 16). As shown in Fig. 1A, all of the CD1 Ptgs2−/− mice (n = 19) ovulated with numbers of ova comparable with those of wild-type females (n = 7). In addition, an improvement in the fertilization rate in CD1 mutant mice was noted, albeit at a reduced level (Fig. 1A). An average of five two-cell embryos was recovered in 14 of 19 (74%) CD1 Ptgs2−/− mice. Although this is lower than the rate in wild-type mice, it is much higher than the rate in C57BL/6J/129 mutant mice observed previously (7, 16). A similar profile of ovulation and fertilization was observed in these null mice after superovulation. However, the cause of this reduced number of two-cell embryos remained unclear. We surmised that reduced numbers of two-cell embryos was the result of reduced fertilization rate in CD1 Ptgs2−/− null females because of defective oocyte maturation and/or aberrant coupling of the cumulus-oocyte complex.

Thus, we examined the status of ovulated eggs on day 1 of pregnancy in CD1 Ptgs2−/− mice. Consistent with the observation of results recorded on day 2, all of the Ptgs2 null mice examined (n = 11) ovulated, and 35% (4/11) of the oocytes were fertilized as indicated by newly formed male and female pronuclei, although a number of Ptgs2 null oocytes showed signs of degeneration or fragmentation (Fig. 1B). These results suggested that failure of some oocytes to undergo fertilization was because of their inability to resume meiosis and subsequent maturation during ovulation. In addition, failure of sperm penetration and sperm-egg fusion was frequently observed in CD1 Ptgs2−/− mice (Fig. 1C). Because Ptgs2 null males are fertile, we speculated that functional deficiency of

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H. Wang and S. K. Dey, unpublished results.

TABLE I

Term pregnancy occurs in CD1 Ptgs2-deficient mice

| Genotype     | No. of sperm-positive mice | No. of mice with term pregnancy | Litter size |
|--------------|----------------------------|-------------------------------|-------------|
| Wild-type    | 10                        | 10 (100%)                     | 14.5 ± 0.8  |
| CD1 Ptgs2−/− | 44                      | 20 (45%)                      | 3.7 ± 0.8  |

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either oocytes or of somatic cumulus cells led to fertilization failure. CD9, a cell surface molecule, is necessary and a marker for normal sperm and oocyte membrane fusion during fertilization (22). We observed normal localization of CD9 in Ptgs2 null oocytes (Fig. 1D), eliminating the defective sperm-egg fusion as the cause of reduced fertilization. Thus, deficient cumulus cell function under Ptgs2 deficiency appears to be a cause for the lower fertilization rate. Indeed, an impaired morphology of the cumulus-oocyte complex with fewer layers of outside cumulus cells and altered muciﬁcation was observed in CD1 Ptgs2 null mice (Fig. 1E). Collectively, our results show that ovulation and to considerable extent fertilization are improved with the introduction of CD1 genetic background in Ptgs2 null females.

Differential Spatiotemporal Expression of Ptgs1 and Ptgs2 Correlates with Differential Ovulation and Fertilization in C57BL/6J/129 and CD1 Mice Lackng PtgS2—To examine the cause(s) of improved ovulation in CD1 versus C57BL/6J/129 Ptgs2−/− mice, we next compared in parallel the ovarian expression proﬁles of Ptgs1 and Ptgs2 during ovulation in wild-type mice and Ptgs1 expression in Ptgs2 mutant mice on CD1 and C57BL/6J/129 backgrounds. A standard superovulation regimen using a combination of eCG and hCG protocol was applied to stimulate follicular growth and induce ovulation at a determined time point (16). Under this treatment schedule, ovulation normally occurs between 12 and 14 h after hCG injection. Ovarian sections were processed for in situ hybridization of Ptgs1 and Ptgs2 at different time points after hCG injections.

An interesting differential Ptgs1 and Ptgs2 expression pattern was observed in C57BL/6J/129 and CD1 wild-type ovaries after hCG stimulation. Although very little Ptgs1 or Ptgs2 mRNA was detected at 0 h, with the initiation of the ovulatory process Ptgs2 expression was induced markedly at 4 h in the preovulatory follicles in both strains of mice. Interestingly, this expression in C57BL/6J/129 mouse ovaries persisted through 12 h followed by a dramatic down-regulation at 16 h post-hCG injection, whereas in CD1 mouse ovaries the expression declined dramatically at 8 h approaching 0 h levels (Fig. 2, A and B). However, at 12 h, Ptgs2 expression was again detected, but at a much lower level compared with high Ptgs2 expression in C57BL/6J/129 ovaries (Fig. 2, A and B). After ovulation (16 h), Ptgs2 expression in CD1 ovaries diminished rapidly reminiscent of that observed for C57BL/6J/129 mice (Fig. 2, A and B). Despite the temporal differences in Ptgs2 expression between these two strains of mice during ovulation, its expression was consistently localized to mural and cumulus granulosa cells of mature follicles. Ptgs1 expression in C57BL/6J/129 mice was ﬁrst observed at 8 h at very low levels followed by a remarkable up-regulation at 12 h with slight down-regulation at 16 h post-hCG stimulation. In CD1 ovaries, the induction was evident at 4 h with further up-regulation at 8 h followed by persistent expression through 16 h post-hCG stimulation. In contrast to Ptgs2 expression, the expression of Ptgs1 was always restricted to mural granulosa cells with no detectable signals in cumulus cells. In the absence of Ptgs2, Ptgs1 expression proﬁles at these time points in both strains of mice remained similar to those in wild-type ovaries.

The analysis of ovulation and fertilization together with the expression studies in wild-type and mutant ovaries suggests that the relative contribution of COX-1- or COX-2-derived PGs in ovulation and fertilization depends upon the genetic background of mice. The results also provide evidence that expression of either COX-1 or COX-2 in mural granulosa cells between 4 and 8 h before ovulation is crucial for successful completion of this process, whereas cumulus cell expression of COX-2 is a critical determinant for normal oocyte maturation and fertilization. This is consistent with failure of ovulation and fertilization in C57BL/6J/129 Ptgs2−/− mutant females as opposed to normal ovulation with reduced fertilization rates in CD1 mutant females. Although the general consensus is that
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CD1 Pregnant Mice Exhibit Improved Implantation in the Absence of COX-2—The “window” of implantation is defined as the limited time span during which the implantation-competent blastocyst establishes a reciprocal interaction with the receptive uterus (17, 23). In mice, the uterus becomes receptive on day 4 of pregnancy with the initiation of the attachment reaction around midnight of the same day (20).

In addition to normal ovulation and improved fertilization, successful term pregnancy occurred in 45% of the plug-positive CD1 Ptgs2 null mice with small litter sizes (Table I). This is in sharp contrast to our present and previous observations of complete failure of successful pregnancy in C57BL/6J/129 Ptgs2−/− females (7). However, frequent pregnancy failures (55%) in CD1 Ptgs2−/− mice suggested that uterine defects from the time of fertilization to parturition still persist. We thus investigated whether one cause of this failure was aberrant implantation and/or decidualization in these mice. Increased vascular permeability at the site of blastocysts, as an index of normal implantation, was recorded on days 5, 6, and 8 of the pregnancy by the blue dye method (17). We observed that a significant number of blastocysts implanted in CD1 Ptgs2 null mice, but the timing of implantation (window of implantation) became variable (Fig. 3, A–C). On midmorning of day 5 of pregnancy, an average of about 14 implantation sites (13.5 ± 0.4/mouse) were observed in all 9 wild-type plug-positive mice, whereas only ~3 implantation sites (3.2 ± 0.5/mouse) were detected in 40% (17/42) of the plug-positive CD1 Ptgs2−/− mice. The absence or reduced number of implantation sites was not solely the result of the reduced number of fertilized embryos because many unimplanted blastocysts were recovered after flushing the uteri of null mice which failed to exhibit blue dye reaction on day 5. The apparent morphological appearance of these blastocysts was normal with some of them being zona-encased, zona-free, or in the process of zona dissolution (Fig. 3D).

We surmised that unimplanted blastocysts observed in CD1 Ptgs2−/− uteri on day 5 could implant beyond the normal window of implantation. Indeed, as shown in Fig. 3A, 39 of 51 (76%) CD1 Ptgs2−/− mice showed distinct implantation sites (6.1 ± 0.5) on day 6 of pregnancy, providing evidence that implantation had occurred beyond the normal window. Collectively, these results demonstrate that although a number of blastocysts implant at the normal anticipated time, some of them implant beyond the normal time of implantation in CD1 Ptgs2−/− mice. However, although an average of approximately 6 implantation sites were recorded on day 6 of pregnancy in CD1 Ptgs2 null mice, the litter size at term was smaller, averaging 4 pups/litter (Table I). This suggests that blastocysts that implanted during the normal window progressed to term, whereas those implanted later became the victim of subsequent developmental anomalies, leading to smaller litter sizes. Similar observations have been made in Pla2g4a null mice in which implantation of blastocysts occur beyond the normal window (18). Implantation of blastocysts in CD1 Ptgs2 null mice at the normal or deferred time suggested that one cause of improved implantation was the compensation of COX-2 deficiency by COX-1. Alternatively, COX-derived PGs may not always be necessary for implantation. To address this issue, we next examined the Ptgs1 expression profile in CD1 Ptgs2 null uteri at the sites of blastocyst attachment (blue bands).

Ptgs1 Compensates for the Loss of Ptgs2 during Implantation in CD1 Mice—To see whether COX-1 can offset the deficiency of COX-2, we first compared the expression of Ptgs1 in both CD1 wild-type and null mice during implantation by in situ

Fig. 2. Differential expression profiles of Ptgs1 and Ptgs2 in C57BL/6J/129 and CD1 Ptgs2−/− mouse ovaries during ovulation. A standard superovulation regimen using a combination of eCG and hCG protocol was applied to stimulate follicular growth and induce ovulation at a defined time point. Ovarian sections from C57BL/6J/129 (A) and CD1 (B) wild-type (WT) or Ptgs2−/− mice were processed for in situ hybridization at different time points after hCG injections. Images are shown at 40×.

rather than in C57BL/6J/129 ovaries in which COX-1 expression is very transient (Fig. 2, A and B).

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hybridization. Normally Ptgs2, not Ptgs1, is expressed in the uterus exclusively at the site of blastocyst during implantation. As shown in Fig. 4A, with the initiation of implantation Ptgs2 is expressed in the luminal epithelium and subepithelial stroma cells surrounding those blastocysts showing implantation (blue band) on day 5 in CD1 Ptgs2−/− mice in a pattern resembling the native expression of Ptgs2 in wild-type mice as observed previously (5). On day 6, the expression of Ptgs1 in CD1 Ptgs2 null mice was primarily restricted to the mesometrial luminal epithelium and stroma of the implantation site, again in a manner comparable with Ptgs2 expression in wild-type mice.² The results of immunolocalization² and Western blotting of COX-1 protein are consistent with in situ hybridization results (Fig. 4B). Furthermore, sustained levels of PGI₂, considered important for implantation (19), in CD1 mice missing the Ptgs2 gene also point toward contribution by compensatory up-regulation of COX-1 (Fig. 5, A and B). Collectively, the results suggest that compensatory up-regulation of Ptgs1 in a pattern similar to Ptgs2 offsets the loss of Ptgs2 in initiating the attachment reaction in CD1 Ptgs2 null mice. In contrast, this compensatory phenomenon was not observed in C57BL/6J/129 Ptgs2−/− mice (Fig. 4A) and explains for the total pregnancy failure in these mice. However, C57BL/6J/129 mice lack sPLA₂-IIA because of natural mutation as opposed to CD1 mice that exhibit variable genotypes of sPLA₂-IIA (18, 24). Thus, it is also possible that sPLA₂-IIA in addition to cytosolic PLA₂ contributes to increased arachidonic acid levels for COX-1 in generating comparable levels of uterine PGs in CD1 Ptgs2−/− mice during implantation.

Decidualization Response Is Different between CD1 and C57BL/6J/129 Ptgs2−/− Mice—With the initiation of the attachment reaction between the blastocyst trophoderm and uterine luminal epithelium, stromal cells surrounding the blastocyst undergo extensive proliferation and differentiation into decidual cells. This decidualization process can also be induced experimentally in pseudopregnant uteri by intraluminal infusion of oil (7). Although “on-time” implantation was deferred for a significant number of blastocysts in CD1 Ptgs2−/− mice, the

![Image](http://www.jbc.org/Downloaded_from/)

**Fig. 3. Implantation and postimplantation development in CD1 Ptgs2−/− mice.** A, the number of implantation sites was examined on days 5, 6, 8, and 12 of pregnancy in CD1 wild-type and Ptgs2−/− mice. The numbers within the bars indicate the number of mice with implantation sites/total number of mice (*, p < 0.01; unpaired t test). B, impaired uterine and embryonic growth in CD1 Ptgs2−/− mice. Correlating with the altered window of implantation, an impaired postimplantation growth was observed in CD1 Ptgs2−/− mice (*, p < 0.01; unpaired t test). The numbers within the bars indicate the average weight of implantation sites on day 12 of pregnancy. C, representative photographs of uteri with implantation sites on days 5–12. Note the smaller size and fewer number of implantation sites in CD1 Ptgs2−/− mice. Arrows indicate the blue bands depicting implantation sites on days 5 and 6. Arrowheads indicate the resorbing implantation sites on day 12 in Ptgs2 null uteri. D, a representative photograph of blastocysts that were pooled from two CD1 Ptgs2−/− mice not exhibiting blue bands on day 5 of pregnancy (10 a.m.) is shown. Note zona-encased (red arrowhead) or zona-free (black arrows) blastocysts or blastocysts in the process of zona dissolution (white arrows).
was expressed in the stroma 24 h after intraluminal oil infusion in this strain of mice. Indeed, we observed that Ptgs1
Ptgs2 in the uterine luminal epithelium and subepithelial stromal cells at the
cidualization, 7 of 8 (88%) CD1
Ptgs2 in the absence of COX-2 is not observed in day 5 C57BL/6J/129
Ptgs2 null mice, exhibiting complete failure of decidualization (Fig. 6B). The analysis of COX-1 protein levels
by Western blotting is consistent with the results of in situ
hybridization (Fig. 6C). To provide further evidence for COX-1 compensation of COX-2 deficiency for decidualization in CD1
mice, we used SC560, a COX-1-selective inhibitor (25, 26), to
block COX-1 activity and recorded its effects on decidualization.
Treatments with SC560 dramatically inhibited decidualization in CD1
Ptgs2–/– mice (Fig. 6A). These results further support our proposition that COX-1-derived PGs can compensate
for the deficiency of COX-2 during implantation and decidualization in CD1 mice. These findings are novel and further
indicate that the genetic background is important in diversification of reproductive phenotypes in Ptgs2 null mice.

Wild-type Blastocysts Transferred into CD1 Ptgs2–/– Uteri Show Variable Timing of Implantation—To dissect out the embryonic versus uterine effects on the timing of implantation, embryo transfer techniques were employed. Wild-type day 4 blastocysts were transferred into day 4 pseudopregnant wild-type or Ptgs2 null mice, and implantation sites were visualized on days 5 and 6 by the blue dye method (17). In CD1 mice, although 54% (38/70) of the transferred blastocysts implanted in all 5 wild-type recipients on day 5, only 14% (10/72) of the transferred blastocysts showed signs of implantation in 2 of 6 mice (33%) CD1 Ptgs2–/– recipients (Fig. 7A). Recovery of 20 blastocysts from these null uteri indicated successful blastocyst transfer but failure of the attachment reaction. However, when we examined the blue reaction 48 h after embryo transfer (day 6), 47% (51/108) of the transferred blastocysts showed implantation in all 8 CD1 Ptgs2–/– mice (Fig. 7A). Furthermore, as shown in Fig. 7B, the size of the implantation sites and intensity of blue bands in Ptgs2 null uteri were variable, indicating different timing of implantation of blastocysts. As expected, the treatment with SC560 on the day of blastocyst transfer and on day 5 drastically reduced the implantation rate in CD1 Ptgs2 null mice when examined on day 6; only 8 of 86 blastocysts showed signs of implantation. These results again provide evidence that up-regulated COX-1 activity can offset the loss of COX-2 in CD1 mice.

Because a temporary delay of implantation was often observed in CD1 Ptgs2 null recipients, we subsequently investigated whether this altered window of implantation could affect the postimplantation embryonic growth. Indeed, although only 8% (3/38) of the implantation sites in wild-type recipients showed signs of resorption, 61% (36/59) of the implantation sites showed signs of resorption in CD1 Ptgs2–/– recipients when examined on day 12 (Fig. 7, A and C). Most of these retarded implantation sites were smaller in size and filled with blood (Fig. 7C); no healthy looking embryos were isolated from these resorption sites. It is to be recalled that similar defective postimplantation growth was also observed when CD1 Ptgs2–/– mice were examined on day 12 after natural mating (see Fig. 3C).

To compare in parallel whether similar events occur in C57BL/6J/129 mice, we again employed blastocyst transfer experiments in C57BL/6J/129 Ptgs2–/– mice and reexamined implantation and postimplantation development as reported previously (7). We observed that although 48% (47/97) of the transferred wild-type blastocysts implanted in 83% (5/6) of the wild-type recipients on day 5, less than 1% of the transferred blastocysts (2/214) showed weak signs of implantation in 2 of 13 C57BL/6J/129 Ptgs2–/– recipients (Fig. 7A). 60 unimplanted blastocysts were recovered after flushing those uteri not showing blue reaction, providing evidence for successful transfers. Furthermore, when examined on day 6, only 5% (15/274) of the transferred blastocysts showed weak blue reaction in 5 of 18
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C57BL/6J/129 \( \text{Ptgs2}^{-/-} \) recipients (Fig. 7A). In recipient mice sacrificed on day 12, again only 6% (8/133) of the transferred blastocysts showed signs of implantation sites that were undergoing resorption; no embryos were present in 7 of 8 resorption sites, and only one site had a very small embryo (Fig. 7C). These results support our previous observation of total failure of pregnancy in C57BL/6J/129 \( \text{Ptgs2}^{-/-} \) null mice (7). Collectively, our findings provide strong evidence that COX-2 has an essential role in implantation and decidualization in C57BL/6J/129 mice (7, 19). This deficiency can be improved significantly by compensatory up-regulation of \( \text{Ptgs1} \) depending on the genetic background.

DISCUSSION

Recent development of COX-2-selective inhibitors and their worldwide use for the alleviation of inflammatory diseases with reported variable efficacy provided us the motivation to explore whether genetic disparity plays any role in altering COX-2 functions. Our observations of normal ovulation with improved fertilization and implantation in CD1 mice in the absence of COX-2 suggest that the efficacy of COX-2-selective inhibitors and other side effects could be a function of genetic diversity also in human populations with different ethnic and racial backgrounds. Although specific gene mutations produce different phenotypes in the context of genetic background in mice (11–14), the underlying molecular mechanisms remain largely unknown. It is often argued that background-dependent altered phenotypes are the result of participation of modifier genes. However, only a few studies have reported the identification of modifier genes in regulating background-dependent phenotypes (10, 12). We propose that a compensatory gene function is an alternative mechanism by which phenotype diversification is achieved in the context of genetic disparity. This proposal is consistent with our present findings that the background-dependent altered phenotypes are the result of differential spatiotemporal expression of COX isoforms in the target tissues and rescue of COX-2 deficiency by COX-1, which functions as an inducible isoform in a fashion similar to native COX-2. Our results also demonstrate for the first time the differential role of ovarian cell-specific COX isoforms in ovulation and fertilization.

The consensus has been that COX-2, but not COX-1, is crucial to ovulation (3, 4). Our observations of failure of ovulation in C57BL/6J mice, but normal ovulation in CD1 mice, in the absence of COX-2 correlate well with differential ovarian expression of \( \text{Ptgs1} \) and \( \text{Ptgs2} \) in these two strains of wild-type mice and of \( \text{Ptgs1} \) in the absence of \( \text{Ptgs2} \). This provides evidence that either COX-1 or COX-2 in mural granulosa cells can replace each other in the process of ovulation, if expressed appropriately. In contrast, our observation of cumulus cell expression of COX-2, but not COX-1, is consistent with the speculation that COX-2 is more important for cumulus cell function (4, 27). The complete lack of fertilization in C57BL/6J/129 mice and somewhat reduced fertilization rate in CD1 mice in the absence of COX-2 again support that COX-2 of cumulus cell origin is an important contributing factor in promoting oocyte maturation and competence to fertilization, although timely expression of granulosa cell COX-1 prior to ovulation can partially rescue fertilization failure as seen in CD1 \( \text{Ptgs2} \) mutant females. This is consistent with the finding that cumulus cell-oocyte interactions are important for the generation of fertilization-competent eggs (28). Collectively, our results of genetic and expression studies demonstrate for the first time the relative contribution of granulosa and cumulus cell COX isoforms in ovulation and fertilization. Although the mechanism of differential COX isoform regulation in C57BL/6J/129 and CD1 ovaries is not known, there is now evidence that multiple quantitative trait loci control strain differences in the ovulation rate (29). It is conceivable that the reproductive traits are different between C57BL/6J/129 and CD1 mice. Nonetheless, we demonstrate a genetic background-dependent functional role of COX-1 in ovulation \textit{in vivo} in the absence of COX-2.

Spatiotemporal expression of COX-1 and COX-2 in the peri-implantation mouse uterus suggests that COX-derived PGs participate in uterine preparation and implantation (5). \( \text{Ptgs1} \) is expressed in the luminal epithelium along the entire uterus on days 3 and 4 but is down-regulated by the afternoon of day 4 prior to the initiation of implantation. In contrast, \( \text{Ptgs2} \) is expressed specifically in the luminal epithelium and stroma immediately surrounding the blastocyst at the time of its attachment with the luminal epithelium on day 4 midnight (5). This expression pattern is common to both C57BL/6J/129 and CD1 wild-type mice. Although uterine COX-2 is essential for implantation in C57BL/6J/129 mice, a unique compensatory up-regulation of COX-1 in the uterus at the site of the blasto-
cyst in CD1 mice lacking COX-2 provides strong evidence that COX-1 is also inducible in a fashion similar to that of COX-2 within a specific genetic environment and contributes to improved implantation in CD1 mice compared with complete failure of implantation in C57BL/6J/129 mice missing Ptgs2.

Although COX-2 deficiency can be compensated by COX-1, COX-2 is still an important factor for the full restoration of female reproductive functions. This is exemplified by the shift-}

ging of the normal window of implantation for certain blasto-
cysts in CD1 mice missing the Ptgs2 gene, leading to subsequent developmental anomalies and small litter sizes. This observation is similar to our previous findings in Pla2g4a-deficient mice (18). These two studies together put forward a new concept that a short delay in the initial attachment reaction propagates detrimental effects during the later course of pregnancy. This is clinically important as implantation beyond the normal window of uterine receptivity leads to increased pregnancy losses in women (30).

In conclusion, our study provides direct in vivo evidence that a compensatory mechanism via COX-1 up-regulation substanti-}
alizes COX-2-deficient female infertility in a genetic background-dependent manner. Considering the worldwide use of nonsteroidal anti-inflammatory drugs including selective COX-2 inhibitors and genetic disposition to drug responses.
Fig. 7. Implantation of wild-type blastocysts transferred into pseudopregnant wild-type or Ptgs2-deficient mice. A, implantation occurred in CD1 Ptgs2<sup>−/−</sup> uteri in a deferred manner, which led to defective postimplantation development. The numbers within the bars indicate the number of mice with implantation sites/total number of mice examined (upper panels) and the number of blastocysts implanted/total number of blastocysts transferred (lower panels) (*, p < 0.01; unpaired t test). *, in C57BL/6J/129 Ptgs2<sup>−/−</sup> recipients, all eight implantation sites were resorbed. In CD1 mice (b) only 3 of 38 (8%) implantation sites in wild-type recipients showed signs of resorption, whereas (c) 36 of 59 (61%) implantation sites showed signs of resorption in Ptgs2<sup>−/−</sup> uteri. B, representative photograph of uteri with implantation sites after blastocyst transfer. Note variable intensity of blue reaction as prominent (red arrowheads) or weak (black arrowheads), indicating different timing of implantation of transferred blastocysts. C, a composite photograph of uteri in wild-type (WT) and Ptgs2<sup>−/−</sup> mice on day 12 of pregnancy after blastocyst transfers. Resorption sites were often noted (arrowheads) in CD1 Ptgs2 null mice. The size and gross morphology of embryos isolated from normally growing implantation sites were comparable between CD1 wild-type and Ptgs2<sup>−/−</sup> mice, whereas only one severely retarded embryo was recovered from C57BL/6J/129 Ptgs2<sup>−/−</sup> recipients.
(15, 31), our present work raises a cautionary note against therapeutic use and efficacy of COX-2 inhibitors in inflammatory diseases among human populations without regard for genetic and ethnic diversities. There is now evidence for the association of aspirin resistance and genetic polymorphisms (31). Thus, the use of these drugs should be evaluated carefully based on genetic disparity with respect to their efficacy and harmful side effects, including their consumption during pregnancy (32, 33).

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