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Assisted Reproduction

Influence of the Preimplantation Embryo Development (*Ped*) Gene on Embryonic Platelet-Activating Factor (PAF) Levels

Elissa T. Purnell,[1] Carol M. Warner,[2] Hilton I. Kort,[3] Dorothy Mitchell-Leef,[3] Carlene W. Elsner,[3] Daniel B. Shapiro,[3] Joe B. Massey,[3] and William E. Roudebush [3,4,5]

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***Purpose*:** A major gene responsible for the control of preimplantation cleavage rate is the *Ped* gene, the product of which is the Qa-2 protein. Fast, but not slow developing mouse embryos express the Qa-2 protein. Platelet-activating factor (PAF) is a novel and potent signaling phospholipid that has unique pleiotropic properties in addition to platelet activation. PAF plays a significant role in virtually every reproductive event, including ovulation, fertilization, implantation, and parturition. The role of the *Ped* gene in PAF production by preimplantation embryos is yet to be established. The presence of this gene provides embryos with a reproductive advantage over those that are *Ped* negative, and may also serve as a regulator of PAF synthesis. The study hypothesis is that the amount of PAF produced is dependent upon the presence or absence of the *Ped* gene.
***Methods*:** B6.K1 (*Ped* negative) and B6.K2 (*Ped* positive) mouse embryo-conditioned culture media were assayed for PAF content by a PAF-specific radioimmunoassay.
***Results*:** There was a significant ($p < 0.001$) difference in blastocyst development rates between the *Ped*+ B6.K2 (61.0%) and the *Ped*− B6.K1 (25.3%) embryo culture groups. There was a significant difference ($p < 0.05$) in PAF production between the *Ped*+ B6.K2 (4.70 ± 0.46 pmol per embryo) embryo culture group and the *Ped*− B6.K1 (10.02 ± 3.49 pmol per embryo) embryo group. The B6.K1 (*Ped*−) embryo group produced >2× more PAF than did the B6.K2 (*Ped*+) group.
***Conclusions*:** The *Ped* gene plays a role in PAF production and release in preimplantation stage embryos. The use of two mouse identical strains, except for the *Ped* gene, show that its presence is associated with an increase in developmental potential. Embryos where the *Ped* gene was absent produced significantly higher levels of PAF, which may aid in their survival.

KEY WORDS: Embryo; mouse; PAF; *Ped* gene.

INTRODUCTION

Preimplantation stage embryos from many mammalian species develop at different rates (i.e., fast or slow). The rate of development is influenced by both genes and the environment. At the genetic level, both maternal and embryonic genes control preimplantation embryo development. In the mouse, the difference in cleavage division rates between slow and fast developing mouse strains is maintained

[1] Savannah State University, Savannah Georgia.
[2] Northeastern University, Boston Massachusetts.
[3] Reproductive Biology Associates, Atlanta Georgia.
[4] Reproductive Biology Associates, 1150 Lake Hearn Drive, Suite 400, Atlanta Georgia 30342.
[5] To whom correspondence should be addressed at Reproductive Biology Associates 1150 Lake Hearn Drive, Suite 400, Atlanta Georgia 30342; e-mail: roudebush@rba-online.com.

in vitro (1) and is strain dependent (2, 3). A major gene responsible for the control of preimplantation cleavage rate is the *Ped* gene, the product of which is the Qa-2 protein (4, 5). Fast, but not slow developing mouse embryos express the Qa-2 protein (6). The embryonic *Ped* gene phenotype is an intrinsic property of the embryos themselves (1). The *Ped* gene also has a role in blastocyst differentiation (7). The product of the *Ped* gene, the Qa-2 protein, is a mouse major histocompatibility complex (MHC) class Ib protein encoded by two almost identical genes (one nucleotide difference), *Q7* and *Q9* (6,8). However, other genes, in addition to the MHC encoded *Ped*, gene, play a role in the control of the rate of early preimplantation embryonic cleavage (9). Mouse strains (B6.K1 and B6.K2) that differ in only the *Ped* phenotype suggest that presence of Qa-2 protein (*Ped* fast) confers a reproductive advantage (10). Birth weights are higher in pups that result from mating B6.K2 (*Ped* positive) mice. Larger litters are observed from B6.K2 females, regardless of the *Ped* phenotype of the males. Progeny resulting from crosses between B6.K1 (*Ped* negative) males and females of either *Ped* phenotype, grew faster than did pups from B6.K2 males crossed with females of either phenotype. Differences in gestational periods have been reported between *Ped* positive and *Ped* negative mice, with B6.K1 displaying longer gestation (11). In addition, B6.K1 mice develop high blood pressure later in life compared to B6.K2 mice (12).

Platelet activating factor (1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphocholine; PAF) is an acetylated glycerophospholipid that was first reported in 1972 (13). This compound is a potent signaling phospholipid that is produced by and influences numerable cell types. PAF is involved in a number of reproductive and developmental processes (14, 15), including ovulation (16), fertilization (17), preimplantation embryo development (18), implantation (19), and parturition (20). Embryos from a number of species produce PAF (15,21,22). PAF is an important mediator of interactions between the embryo and the endometrium (22). A failure of these interactions to occur may cause infertility. PAF is produced by uterine tissue and preimplantation stage embryos. PAF levels in the rabbit uterus increase from day 3 to day 5 of pregnancy (20). Embryonic PAF in the rabbit and mouse increases during the preimplantation phase, with maximum levels at the expanded blastocyst stage (15,22). Embryo-derived PAF is essential for implantation and the establishment of pregnancy (23, 24). Human preimplantation embryos synthesize and secrete PAF (21) and PAF is considered a biomarker indicating the likelihood of pregnancy (25). Additionally, embryonic-PAF has been suggested to play a survival factor role (26).

The role of the *Ped* gene in PAF production by preimplantation embryos has yet to be established. The presence of this gene provides embryos with a reproductive advantage over those that are *Ped* negative, and may also serve as a regulator of PAF synthesis. The study hypothesis is that the amount of PAF produced is dependent upon the presence or absence of the *Ped* gene.

MATERIALS AND METHODS

The care and use of the animals described herein were approved by the Institutional Animals Care and Use Committee (IACUC, Northeastern University). Mice were housed in a viral-free facility with a 14 h:10 h light:dark photoperiod at 23°C and food and water provided ad libitum. The B6.K1 (*Ped* negative) and B6.K2 (*Ped* positive) mouse strains were derived from H-2/Tla crossovers, which occured in C57BL/6 × A mice (27). Two-cell embryos were collected from the excised oviducts of pregnant mare's serum gonadotropin and human chorionic gonadotropin (PMSG and hCG; 5 IU each; Sigma) primed B6.K1 and B6.K2 females (4 weeks of age) mated with fertile males (6 months of age) of the appropriate strain. The interval between PMSG and hCG was 46–48 h. Embryos were cultured (25 embryos/50 μL microdrop) in modified M16 under sterile-equilibrated mineral oil in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C, to the blastocyst stage. Following culture, the embryo-conditioned culture media were recovered and treated with an equal volume of 20% glacial acetic acid. The samples were frozen at −70°C and shipped on dry-ice for PAF analysis.

Embryo-conditioned media were assayed for PAF content by using a PAF-specific radioimmunoassay (^{125}I) according to the manufacturer's instructions (NEN Research Products; DuPont, Boston, MA). Primary antibodies were added to tubes, which contained embryo-conditioned media or assay buffer. The tubes were mixed and incubated for 15 min at room temperature. Secondary antibodies conjugated with ^{125}I labeled PAF tracer were added and the samples, standards, blanks, and total count tubes, mixed,

Table I. Effect of the *Ped* Gene on Blastocyst Development

Mouse strain	*Ped* phenotype	Blastocyst development rate
B6.K2	Qa-2 positive	61/100 (61.0%)*
B6.K1	Qa-2 negative	38/150 (25.3%)*

*$p < 0.001$.

and incubated at room temperature. Following a 24 h incubation period, tubes were centrifuged ($2000 \times g$; 30 min), supernatants decanted and the tubes blotted and counted in a gamma counter (Packard Instrument Company, Downers, IL) for 1 min. The standard curve was calculated by regression analysis (logit value of normalized percent bound versus log of nanograms of PAF assayed). Content of extracted PAF is expressed as pmol per embryo.

Data were evaluated by Student's *t* test and 2×2 chi-squared contingency table.

RESULTS

A total of 100 B6.K2 (*Ped* +) and 150 B6.K1 (*Ped* −) preimplantation stage embryos were collected and cultured to the blastocyst stage as described. Embryo-derived PAF was detected in all B6.K1 (*Ped* −) and B6.K2 (*Ped* +) embryo-conditioned culture media. Blastocyst development rates and embryo-derived PAF levels in B6.K1 (*Ped* −) and B6.K2 (*Ped* +) embryo-conditioned culture media are provided in Tables I and II, respectively. There was a significant ($p < 0.001$) difference in blastocyst development rates between the *Ped* + B6.K2 (61.0%) embryo culture group and the *Ped* − B6.K1 (25.3%) embryo culture group (Table I). There was a significant difference ($p < 0.05$) in PAF production between the *Ped* + B6.K2 (4.70 ± 0.46 pmol per embryo) embryo culture group and the *Ped* − B6.K1 (10.02 ± 3.49 pmol per embryo) embryo group (Table II). The B6.K1 (*Ped* −) embryo group produced >2 × more PAF than did the B6.K2 (*Ped* +) group (Table II).

Table II. Effect of the *Ped* Gene on Embryonic-PAF Production

Mouse strain	*Ped* phenotype	PAF production (pmol per embryo)
B6.K2	Qa-2 positive	4.70 ± 0.46*
B6.K1	Qa-2 negative	10.02 ± 3.49*

*$p < 0.05$.

DISCUSSION

Although the B6.K1 embryos (*Ped* negative) lack the *Ped* gene, they produced more PAF into the culture medium as compared to the B6.K2 embryos (*Ped* positive). It appears that the *Ped* negative mice strain produce twice as much PAF, though a wider range of variability in PAF production was noted in this strain. Another point of interest was the rate at which the *Ped* negative strain developed to the blastocyst stage. At least 75% of the B6.K1 embryos failed to become blastocysts during the culture period. In all probability, the *Ped* − embryos that developed to the blastocyst stage may have markedly upregulated PAF production in order to survive (26). Therefore, a relationship appears to exist between PAF and the absence or presence of the *Ped* gene.

A number of studies have analyzed the production of PAF by mouse embryos (22,28,29). Ryan *et al.* (28) reported an increase in PAF production by two-cell Quackenbush embryos up to a period of 6 h. The total culture time did not extend past 24 h for each stage of the preimplantation embryo development tested. Embryos cultured in groups of 10, 20, 30, and 45 averaged 2.4 pmol of PAF per embryo per 24 h period. This group also looked at in vivo PAF production by morula and blastocyst stage embryos collected fresh from the reproductive tract. The calculated values for the freshly collected embryos were similar to the values obtained for two-cell embryos, suggesting that reduced PAF synthesis in vitro was a result of culture and not the progression of the developmental stages.

The rate of early embryo development in the mouse was originally linked to the MHC in the early 1980s (30). Genetic analysis of five strains of mice demonstrated that slow development was associated with the H-2^k haplotype, while fast development was associated with the H-2^b haplotype. Fast development was dominant. The more slowly developing embryos displayed fewer cells than the fast developing embryos during the preimplantation period. The differences in the rate of development were such that embryos from mice with the H-2^k haplotype were morulae when embryos with the H-2^b haplotype were blastocysts.

Further genetic analysis showed that the *Ped* gene maps to the Q region of the MHC (31, 32). It was suggested that Qa-2 protein, encoded on the Q region, was the *Ped* gene product (33). The majority of the previous studies into the *Ped* gene were conducted using embryos that developed in vivo. To determine

whether *Ped* gene expression was a result of the uterine environment or an intrinsic property of the embryos, Brownwell and Warner (1) investigated the effects of the *Ped* gene on in vitro culture. Their results showed that the *Ped* gene phenotype was maintained in culture, and that the regulation of cleavage in early embryonic development is, therefore, dependent upon embryonic genes.

Tian *et al.* (34) demonstrated that the *Ped* gene phenotype of an embryo could be changed. This was accomplished by treating glycosylphosphatidylinositol (GPI)-linked forms of membrane bound Qa-2 antigen with phosphatidylinositol phospholipase C (PI-PLC). The GPI-associated form is susceptible to cleavage by PI-PLC. The removal of the PI-PLC responsive molecule changed the *Ped* gene phenotype from fast to slow, further suggesting that the Qa-2 protein is the product of the *Ped* gene. Conversions from one *Ped* phenotype to the other were also reported to occur when strains where the *Q7*/*Q9* genes were missing were injected with the genes taken from a *Ped* fast mouse strain (6,8), proving that the *Ped* gene product is Qa-2 protein.

A relationship exists between the *Ped* gene and platelet-activating factor. Slow developing embryos produce more PAF than do fast developing embryos. Both maternal and embryonic genes genetically control preimplantation embryo development. Mouse embryonic-PAF synthesis is influenced by the presence of the *Ped* gene. This project is the first to report on the possible role of the *Ped* gene in PAF production. Additional studies are warranted to further elucidate the relationship between PAF and the *Ped* gene on preimplantation embryo development.

CONCLUSIONS

The *Ped* gene plays a role in PAF production and release in preimplantation stage embryos. The use of two mouse strains identical, except for the *Ped* gene, shows that its presence is associated with an increase in developmental potential. Embryos where the *Ped* gene was absent produced significantly higher levels of PAF, which may aid in their survival.

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