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Implantation, deciduoma formation and live births in mast cell-deficient mice (W/W^v)

R. J. Wordinger, F. L. Jackson and A. Morrill

Department of Anatomy, North Texas State University, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107, U.S.A.

Summary. The intraluminal injection of oil produced deciduoma formation in ovariectomized, mast cell-normal (+/+) and mast cell-deficient (W/W^v) mice that were treated with exogenous steroids. Oil injection and trauma (e.g. sutures) also produced a deciduoma in ovariectomized +/+ and W/W^v mice that had received a single control (+/+) ovary transplanted under the kidney capsule. After transfers of donor blastocysts, implantation and live births were obtained in +/+ and W/W^v mice containing a single ovary transplant. Our results demonstate that uterine mast cells are not required for the production of a decidual cell response, implantation, gestation or the birth of live offspring in mice.

Introduction

The role of histamine in implantation, the formation of a decidual cell response, gestation and parturition has been debated for many years without resolution. Shelesnyak (1957, 1960) proposed that endometrial histamine was involved in decidualization while Nalbandov (1971) reported that histamine was required for implantation. Kahlson & Rosengren (1971) have suggested that histamine was involved in the initiation of parturition via control of uterine contractility. In contrast to these reports, DeFeo (1967) has contended that histamine is not involved in these reproductive processes.

However, most of these studies were primarily based on the assumption that the source of the physiologically significant histamine was uterine mast cells. The existence of a non-mast cell uterine pool and an embryonic pool was not known. These various sources of potentially significant histamine are difficult to isolate or manipulate experimentally with any assurance.

In 1978, Kitamura, Go & Hatanaka reported that the WBB6F-W/W^v inbred strain of mouse was mast cell-deficient. We have shown that the W/W^v mouse uterus is devoid of stainable mast cells and contains low endogenous levels of uterine histamine (Wordinger, Orr, Pace & Brown, 1983). These results make the W/W^v mouse suitable for studying the role of uterine mast cells and histamine in reproductive processes. It has also been demonstrated that W/W^v mice are sterile because of a defect in germ cell migration (Russell, 1954). The ovaries of W/W^v mice are atrophic with an absence of follicles and distinct corpora lutea but contain hyperplastic stroma (Wordinger, Orr, Pace, Oakford & Morrill, 1985). Therefore, to use W/W^v mice to study the role of the various histamine pools in reproduction, the animals must be ovariectomized and provided with exogenous sex steroids or a functioning ovarian transplant.

The objectives of this series of experiments were (1) to determine whether a deciduoma could be elicited in ovariectomized, hormonally treated W/W^v mice by using a physiological inducer (e.g. intraluminal oil injection), (2) to determine whether ovariectomized +/+ and W/W^v mice containing a single ovarian transplant would respond after trauma (e.g. sutures) or oil as inducers with the formation of a deciduoma, and (3) to transfer donor blastocysts into pseudopregnant +/+ and

W/W^v mice containing an ovarian transplant and determine whether implantation and live births occur in the absence of uterine mast cells.

Materials and Methods

Animals. Female WBB6F-W/W^v mice and normal female littermates (WBB6F-+/+) were purchased at 4–6 weeks of age from the Jackson Laboratory, Bar Harbor, ME. Animals were housed in groups of 6 with food and water *ad libitum*. A light regimen of 14 h light:10 h dark and a constant temperature of 23°C were maintained.

Ovariectomy and hormone treatment. At 1–2 weeks before the start of the hormone injections, +/+ and W/W^v mice under general anaesthesia (Metofane: Pitman-Moore Inc., Washington Crossing, NJ) were bilaterally ovariectomized by a dorsolateral approach. After 2 weeks, a steroid hormone injection schedule that had previously been shown to sensitize the uterus for implantation and a decidual cell response (Rankin, Ledford, Jonsson & Baggett, 1979) was begun: Days 1–3, 0·1 μg oestradiol-17β; Days 4 and 5, no treatment; Days 6 and 7, 6·7 ng oestradiol + 1·0 mg progesterone; Days 8–11 1·0 mg progesterone. All injections of hormones were given subcutaneously in 0·1 ml corn oil at 09:00 h. Control animals received 0·1 ml corn oil without hormones.

Ovariectomy and ovary transplantation. Bilateral ovariectomy of +/+ and W/W^v mice was performed as described above. At the same time, the kidney was exposed and a small incision was made in its fibrous capsule. A blunt glass probe was inserted to create a space under the capsule and a single ovary from a +/+ donor animal was inserted into the space. Animals were allowed to recover for 2–3 weeks before deciduoma induction or embryo transfer.

Decidualization induction. At 3 days after mating, the uterine horns of ovariectomized, hormonally-primed W/W^v and +/+ mice and of ovariectomized, ovary-transplanted W/W^v and +/+ mice were stimulated to produce an artifical decidual cell response. For trauma induction, one uterine horn was exposed and three sutures (4–0, silk; Ethicon, Somerville, NJ) were placed at equal distances through the wall of the uterine horn. For oil induction, one uterine horn was exposed and 0·01 ml arachis oil was injected through a 25-gauge needle into the uterine horn. For both trauma and oil injection, the contralateral uterine horn served as a non-stimulated control horn and received neither sutures nor oil.

Superovulation and blastocyst recovery. Superovulation was induced in ICR outbred mice (Harlan Sprague–Dawley, Indianapolis, IN) by i.p. injection of 5 i.u. PMSG (Gestyl, Organon Inc., East Orange, NJ) followed 48 h later by 5 i.u. hCG (Carter-Glayau Laboratories, Glendale, AZ). Injected mice were caged overnight with male mice of proven fertility and mating was verified the following morning by the presence of a vaginal copulatory plug. At 3·5–4·0 days after mating, blastocysts were recovered by flushing each excised uterine horn with 0·5 ml medium. This medium consisted of CMRL-1066 (Flow Laboratories, McLean, VA) plus 20% heat-inactivated fetal calf serum (Flow Laboratories) and antibiotics. Visibly normal blastocysts were pooled in culture medium in a continuous-flow atmosphere of 95% air plus 5% CO_2 until transferred to recipient animals. Culture time never exceeded 15 min.

Embryo transfer. Ovariectomized +/+ and W/W^v mice containing a transplanted ovary were made pseudopregnant by mating with proven males and verified by the presence of a vaginal plug. After 72 h the mice were given a general anaesthetic (Metofane: Pitman-Moore Inc., Washington Crossing, NJ) and the left uterine horn was exposed through a lateral flank incision. A 22-gauge thin-wall multiple-sample vacutainer needle (Becton-Dickinson, Rutherford, NJ) was inserted into the uterine lumen at the cranial end of the uterine horn. The needle served as a trocar for the insertion of a finely drawn 22·5 cm Pasteur pipette which contained 8 donor blastocysts and ~0·5 μl

culture medium. The pipette was inserted for the length of the trocar so that the tip of the pipette was even with the tip of the trocar. The trocar was removed from the uterine lumen and gently moved up the shaft of the pipette to reveal the meniscus of the culture medium. The pipette was allowed to remain in the uterine lumen. By using mouth pressure, the blastocysts and a small amount of culture medium were expelled into the uterine lumen. Care was taken not to expel air into the uterine lumen, since air has been shown to cause a decidual cell response (Orsini, 1963). The pipette was then removed from the uterine horn. No bleeding or leakage of medium from the uterine horn was observed. The emptied pipette was examined under a dissecting microscope to ensure that no blastocysts remained in the pipette.

Tissue preparation for light microscopy. Samples of uterine horn tissue were obtained 72 h after embryo transfer. All tissue samples were fixed in 4% neutral buffered formalin (pH 7·3) for 48 h and then processed in an automatic tissue processor, embedded in a paraffin wax–paraplast mixture (1:1 v/v) and sectoned at 5 μm on a rotary microtome. Representative sections were stained with haematoxylin and eosin and examined with a Zeiss Photomicroscope III using bright field illumination.

Statistical analysis. The uterine deciduoma data are presented as the means ± the standard error of the mean. Student's *t* test was used for comparisons between two groups. Differences were considered significant at $P < 0.05$ and $P < 0.01$.

Results

Ovarian transplantation

Evaluation of ovarian transplants was made via light microscopy. No difference was observed between transplanted ovaries into +/+ or W/W^v mice. The transplanted ovary remained on the convex surface of the kidney and was surrounded and held in place by the kidney capsule. Distinct ovarian follicles in various stages of growth and development and individual corpora lutea were observed. Oestrous cycles were verified in all animals by means of vaginal smears and mating to males was confirmed by the presence of vaginal plugs. No areas of lymphocyte invasion into the ovarian transplant or other signs of tissue rejection were noted.

Decidual cell response

As shown in Table 1 the injection of 0·01 ml arachis oil into the endometrial lumen produced an extensive deciduoma in the ovariectomized +/+ and W/W^v mice treated with exogenous steroids. There was significant weight increase in the oil-injected uterine horn in comparison to the untouched contralateral control horns. The ability to produce a deciduoma in ovariectomized +/+ and W/W^v mice containing a single ovarian transplant is also demonstrated in Table 1. Intraluminal injection of oil and trauma (e.g. sutures) both produced significant increases in uterine horn weight in comparison to unstimulated contralateral control horns. However, the oil-induced deciduoma was heavier. The difference in weight most probably represents the involvement of the entire uterine horn in an oil-induced deciduoma as opposed to discrete areas around sutures in the trauma-induced deciduoma.

Implantations and live births

Implantation of donor blastocysts and subsequent birth of live young were obtained in the +/+ and W/W^v mice. There was no significant difference between W/W^v and +/+ mice in the implantation rate at 72 h after blastocyst transfer or in the number of live births.

Table 1. Uterine horn weights (mean ± s.e.m.) in ovariectomized +/+ and W/W^v mice after an oil- or trauma-induced decidual cell response

Mouse type	Deciduoma inducer	Steroid source	No. of mice	Uterine weight (mg)	
				Stimulated horn	Unstimulated horn
+/+	Oil	Exogenous	4	272·39 ± 60·4*	39·24 ± 4·5
W/W^v	Oil	Exogenous	5	278·83 ± 22·7*	24·36 ± 2·4
+/+	Oil	Ovarian transplant	4	354·2- ± 20·4**	35·8 ± 3·2
W/W^v	Oil	Ovarian transplant	4	291·4 ± 21·4**	29·9 ± 4·0
+/+	Suture	Ovarian transplant	5	131·30 ± 20·8**	31·7 ± 1·3
W/W^v	Suture	Ovarian transplant	5	157·6 ± 40·1*	20·2 ± 1·5

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ compared with corresponding unstimulated horn.

In one group of animals, implantation was examined histologically at 72 h after blastocyst transfer. In 4 W/W^v mice 19 implantations (63%) resulted from 30 transferred blastocysts while in 2 +/+ mice there were 10 implantations (77%) from 13 transferred blastocysts. Histological examination of the implantation sites at 72 h after transfer revealed comparable decidual cell responses in the +/+ and W/W^v mice. Mesometrial and antimesometrial decidual cells were present. The antimesometrial decidual cells were large and rounded while those close to the developing embryo appeared to be degenerating. The embryo lacked a zona pellucida and differentiation of the germ layers had begun.

In a subsequent group of animals, the birth of live young was assessed. In 3 W/W^v mice, 12 live young (48%) resulted from 25 transferred blastocysts while in 3 +/+ mice there were 15 live young (63%) from 24 transferred blastocysts.

All young survived until weaning at 3 weeks of age. There were no differences between growth rate of young nursed by W/W^v or +/+ mothers.

Discussion

Previous work by Hatanaka, Kitamura, Maeyama, Watanabe & Matsumoto (1982) had shown that ovariectomized W/W^v mice that were treated with exogenous sex steroids would form a deciduoma when trauma (e.g. crushing) was utilized as the inducer. However, Finn & Hinchliffe (1964) have shown that a trauma-induced deciduoma is not physiological since the response is only progesterone-dependent. Implantation in the mouse (Mayer, 1963) and the formation of a deciduoma by the injection of oil into the mouse uterine lumen (Finn & Hinchliffe, 1964) are progesterone- and oestrogen-dependent. Our results using intraluminal oil injection into ovariectomized W/W^v mice demonstrate that uterine mast cells are not required for a decidual cell response that is oestrogen- and progesterone-dependent and thus mimics the requirements for implantation.

Since we were not certain that implantation could occur in ovariectomized mice receiving exogenous steroids, we also used the alternative approach of ovary transplantation. Our results demonstrate that a single ovary transplanted under the kidney capsule re-establishes oestrous cycles in W/W^v mice as determined by vaginal smears and allowed successful mating to occur as determined by the presence of a vaginal copulatory plug. Transplanted ovaries contained ovarian follicles in various stages of development and distinct corpora lutea. The ovaries had established an extensive vascular supply and there were no signs of ovarian dysfunction or tissue rejection.

Ovarian transplantation into ovariectomized W/W^v and $+/+$ mice allowed an extensive decidual cell response to be produced by both trauma (e.g. sutures) or intraluminal oil injection. The oil-induced decidual cell response was more extensive. This reflects the total involvement of the uterine horn in an oil induced deciduoma as opposed to distinct deciduomas around individual sutures. These results demonstrate that (1) progesterone-dependent (e.g. trauma) and (2) oestrogen and progesterone-dependent (e.g. oil) decidual cell responses can be elicited in ovariectomized W/W^v and $+/+$ mice with a single ovarian transplant and that the presence of uterine mast cells is not required. The results obtained with ovarian transplants were comparable to those obtained with exogenous sex steroids.

Using ovariectomized W/W^v and $+/+$ mice containing ovarian transplants as recipients, we obtained successful implantation and the birth of live pups following blastocysts transfer. Histological examination of implantation sites at 72 h after blastocyst transfer revealed no morphological differences between W/W^v and $+/+$ mice. The absence of uterine mast cells in W/W^v mice with an ovarian transplant has been noted previously (F. L. Jackson, unpublished data). These results therefore demonstrate that the early events of embryonic attachment, invasion and implantation do not require the presence of uterine mast cells. Likewise, the birth of live young in W/W^v mice after blastocyst transfer demonstrates that normal gestation and parturition will occur in mast cell-deficient mice. The W^{sh} mutant mouse also lacks mast cells (Loutit, Peters & Stevens, 1981) and has been reported to be fertile (Lyon & Glenister, 1982). In addition, it is apparent that a single ovary, transplanted to the kidney capsule, is sufficient to maintain the endocrine profile required for implantation, gestation and parturition.

The role of the uterine mast cell in reproductive processes has been debated for many years. Using toluidine blue, which stains mast cell heparin, Shelesnyak (1960) and DeFeo (1967) demonstrated that the number of uterine mast cells decreases at the time of implantation. Both Shelesnyak (1957) and Marcus, Shelesnyak & Kraicer (1964) reported that uterine histamine content also decreases at the time of implantation. A decrease in the number of stainable mast cells has been interpreted to indicate that the contents of mast cell granules (e.g. histamine) have been released. Reports of this type have led to the hypothesis that mast cells and/or histamine were involved in the implantation process.

Brandon & Bibby (1979) reported a decrease in mast cell numbers during the attachment phase. However, the decrease occurred both at and between actual attachment sites and did not occur before the Pontamine blue reaction. These results indicate that mast cell degranulation may be a general response. Our present results indicate that, if histamine is involved in implantation, gestation or parturition, its site of origin is not the uterine mast cell.

The existence of a non-mast cell pool of uterine histamine has been reported by Hatanaka *et al.* (1982) and Wordinger *et al.* (1983). This pool of histamine is low and is not significantly altered by ovariectomy or the subsequent administration of exogenous steroids (Wordinger *et al.*, 1985). The presence of a non-mast cell pool of uterine histamine is not a unique finding. Yamatodani, Maeyama, Watanabe, Wada & Kitamura (1982) reported the presence of a similar histamine pool in the brain, stomach, liver, kidney, spleen and skin of W/W^v mice. While the origin of the non-mast cell pool of uterine histamine is unknown, Robinson-White & Beaven (1982) have shown an association of histamine with the endothelial cells of brain capillaries. It is therefore possible that uterine endothelial cells may be the source of this histamine pool. The significance of this histamine pool is not clear at the present time.

The existence of an embryonic histamine pool has been suggested by Dey & Johnson (1980). Although individual mouse blastocysts contain relatively small amounts of histamine (2·0 pg /blastocyst; R. J. Wordinger, unpublished data) it may be sufficient to aid in the initial aspects of implantation and the formation of a decidual cell response. For example, embryonic histamine may be involved in the earliest changes in the endometrial stroma at the site of implantation. These early changes include a localized increase in endometrial vascular permeability as demonstrated by the

Pontamine blue reaction. Embryonic histamine may be involved in this change. Further use of the W/W^v mouse will permit study of the significance of these pools in implantation, decidual cell response, gestation and parturition.

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