An assessment of mast-cell deficient mice (W/W^v) as a model system to study the role of histamine in implantation and deciduoma formation

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Summary. The ovaries from mast cell-normal (+/+) and mast cell-deficient (W/W^v) mice were examined with light and electron microscopy. In addition the effect of ovariectomy and subsequent steroid treatment on total uterine histamine content, total mast cell numbers and surface and glandular epithelial cell heights was measured. The ovaries of +/+ mice were normal, displaying various stages of follicular growth and atresia and numerous corpora lutea; the ovaries of W/W^v mice lacked follicles and corpora lutea but contained numerous hyperplastic interstitial cells which contained numerous lipid droplets, vesiculated mitochondria and abundant endoplasmic reticulum suggestive of steroid synthesis. Steroid treatment of ovariectomized +/+ and W/W^v mice caused a significant increase in uterine wet weight and endometrial surface and glandular epithelial cell heights. In +/+ mice, steroid treatment caused a concomitant increase in total mast cells per uterine horn while mast cells were totally absent in W/W^v mice. The increase in uterine histamine in +/+ mice is consistent with the increase in mast cell numbers. Measurable amounts of uterine histamine, which increases slightly after steroid treatment, were demonstrated in W/W^v mice. Since the uteri of +/+ and W/W^v mice respond to steroids in a similar manner with the sole exception being histamine content and mast cell numbers, our results demonstrate the potential of using these animals to investigate the role(s) of uterine mast cells and non-mast cell uterine histamine in the process of implantation and the formation of a decidual cell response.

Introduction

The successful implantation of the blastocyst into the endometrium and the development of a decidual cell response involves many complex maternal and embryonic interactions. Although various theories have been proposed, the exact mechanisms or factors involved in these processes are not completely understood.

The role of histamine in implantation and stromal differentiation has been debated, without resolution, for the past 25 years. Shelesnyak (1957, 1960) and Nalbandov (1971) have proposed that uterine histamine of mast cell origin is involved in the initiation of implantation and the decidual cell response. Numerous studies concerning (1) changes in mast cell numbers at the time of implantation or in response to steroids (Shelesnyak, 1960; De Feo, 1967; Brandon & Bibby, 1979), (2) uterine histamine content (Marcus, Shelesnyak & Kraicer, 1964) and (3) the effects of various histamine receptor antagonists (Brandon, 1977; Brandon & Wallis, 1977; Brandon & Raval, 1979) have suggested a role for uterine histamine. The initial studies were primarily based on the assumption that the source of this histamine was uterine mast cells. This pool of histamine is difficult to manipulate with assurance and the existence of a non-mast cell pool of

uterine histamine (Hatanaka, Kitamura, Maeyama, Watanabe & Matsumoto, 1982; Wordinger, Orr, Pace & Brown, 1983) and embryonic histamine (Dey & Johnson, 1980) was uncertain and hence not taken into consideration. If the role(s) of these various pools of histamine in implantation and the decidual cell response is to be clarified, a system must be used in which these various sources of histamine can be experimentally manipulated.

Kitamura, Go & Hatanaka (1978) reported that an inbred strain of mouse (WBB6F₁-W/W^v) was mast cell deficient. In addition to mast cell deficiency, a double mutant allele at the *W* locus produces macrocytic anaemia, lack of hair pigmentation and sterility because of a greatly reduced number of oocytes (Russell, 1954) due to an interference with the migration and proliferation of primordial germ cells (Mintz & Russell, 1957). It is unclear whether (1) the ovaries of W/W^v mice are capable of steroid production, (2) the endometria of W/W^v mice will respond to exogenous steroids, (3) the uterus of W/W^v mice is totally lacking in mast cells and (4) W/W^v mice can be experimentally treated with steroids to make them suitable recipients for transferred blastocysts.

The objective of this series of experiments was to examine (1) the morphology of the W/W^v ovary using both light and electron microscopy and (2) the effect of ovariectomy and subsequent steroid treatment on the uterus of mast cell-deficient (W/W^v) mice using congenic mast cell-normal (+/+) mice as controls.

Materials and Methods

Animals. Female WBB6F₁-W/W^v mice and normal female littermates (WBB6F₁-+/+) 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 : dark cycle 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, bilateral ovariectomy was performed under general methoxyflurane anaesthesia by a dorsolateral approach. The ovaries were prepared for light and transmission electron microscopy (see below). After ovariectomy steroid hormone injections were started on a schedule which had previously been shown to sensitize the uterus for implantation and a decidual cell response (Rankin, Ledford, Jonsson & Baggett, 1979); i.e. Days 1–3, 0.1 µg oestradiol-17β; Days 4 and 5, no treatment; Days 6 and 7, 6.7 ng oestradiol-17β + 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.

Light and electron microscopy. At the time of ovariectomy, one ovary was placed in 10% buffered formalin, processed in a tissue processor, embedded in a paraffin wax–paraplast mixture (1 : 1 v/v) and sectioned at 5 µm on a rotary microtome. After staining with haematoxylin and eosin, all slides were examined on a Zeiss Photo III microscope. The other ovary was placed for 1 h in 0.1 M cacodylate-buffered 2% glutaraldehyde (pH 7.2). After a buffer rinse, post-fixation was carried out for 1 h using 1% OsO₄ in the same buffer. Dehydration in a graded ethanol series was followed by clearing in acetone, infiltration, and embedding in Polybed 812 (Polyscience Inc., Warminster, PA). Tissues were sectioned on an ultramicrotome and semi-thin (1 µm) sections were affixed to glass slides and stained with 1% toluidine blue. Thin sections (60–100 nm) were collected on uncoated copper grids and after staining with uranyl acetate and lead citrate examined on an Hitachi H-600 transmission electron microscope.

At the time of death (i.e. Day 7 of steroid treatment) the uterus was immediately dissected and trimmed of fat. The left uterine horn was weighed and stored at –70°C for subsequent histamine assay (see below). The right uterine horn was placed in 10% neutral buffered formalin (pH 7.2), embedded in paraffin wax, serially sectioned at 10 µm and stained with acidified (pH 3.0) toluidine blue for mast cell identification. Mast cells were identified by their large ovoid size (50 µm) and a central nucleus which was often obscured by a dense accumulation of purple metachromatic cytoplasmic granules.

Mast cell counts and endometrial epithelial cell heights. Using $\times 400$ magnification, the number of mast cells contained within the endometrium and myometrium were counted per serial section. Care was taken not to count the same mast cell twice if it appeared on subsequent serial sections. Results are reported as total numbers of mast cells per uterine horn. After mast cell counting, 5 random uterine sections from each experimental and control animal were destained with 100% ethanol and restained with haematoxylin and eosin (H&E) for general histological examination.

To assess the endometrial response to steroids, endometrial surface and glandular epithelial cell heights were measured. Five random measurements from each H&E slide were made with the aid of a calibrated eyepiece micrometer at $\times 400$ magnification.

Histamine assay. The uterine horn to be assayed for total histamine content was homogenized in 500 μ l ice-cold demineralized distilled water by use of a polytron. The homogenate was then heated for 10 min in a boiling water bath to destroy endogenous *S*-adenosyl-*L*-methionine and other heat-labile compounds and to precipitate proteins. After cooling on ice, the heated homogenate was centrifuged at 50 000 *g* for 10 min and 10 μ l samples of the resulting supernatant were assayed for histamine.

Histamine was assayed radioenzymically using a partly purified preparation of rat kidney histamine-*N*-methyltransferase to transfer a tritium-labelled methyl group from [3 H]adenosyl methionine onto histamine (Orr & Quay, 1975; Orr & Eichelman, 1979). The radioactive product of this reaction, [3 H]methyl-histamine, was separated from unreacted [3 H]adenosyl methionine and other radioactive products by ascending thin-layer chromatography (t.l.c.) in a chloroform:methanol:ammonium hydroxide (12:7:1 by vol.) solvent system (Orr & Pace, 1984). The [3 H]methyl-histamine spots (identified with ninhydrin) were scraped from the t.l.c. plate, placed into a plastic scintillation vial and eluted with absolute ethanol (500 μ l). After the addition of toluene phosphor, the radioactivity was determined by liquid scintillation spectroscopy, and, by using internal histamine standards, the levels of histamine in the tissue were determined.

Results

Ovarian morphology

Light microscopy demonstrated that ovaries from $+/+$ mice were normal in that they contained numerous follicles in various stages of growth and atresia as well as morphologically distinct corpora lutea. Ovaries from W/W^v mice were atrophic with a total absence of follicles and distinct corpora lutea (Pl. 1, Fig. 1). The ovarian stroma consisted of many enlarged cells with a pale-staining cytoplasm and nuclei with distinct nucleoli (Pl. 1, Fig. 2). Thick plastic sections stained with toluidine blue revealed numerous lipid droplets within these cells (Pl. 1, Fig. 3).

Electron microscopy of ovaries from $+/+$ mice demonstrated normal follicular elements as well as corpora lutea. Electron microscopy of ovaries from W/W^v mice showed that the cytoplasm of the enlarged stromal cell population consisted of numerous membrane-bound lipid droplets, abundant profiles of smooth endoplasmic reticulum and numerous vesiculated mitochondria (Pl. 2, Fig. 4).

Steroid treatment of ovariectomized mice

As shown in Table 1, steroid treatment of ovariectomized $+/+$ and W/W^v mice caused a significant increase in uterine wet weight. In $+/+$ mice, there was a concomitant increase in total mast cells per uterine horn. These cells were identified as large cells with numerous cytoplasmic granules which are metachromatic after toluidine blue staining. These cells were found throughout the uterus but were more numerous in the myometrium and only occasionally seen in the endometrium. However, no mast cells were found in the uteri of vehicle- or steroid-treated W/W^v mice.

Table 1. Influence of ovarian hormones on various measurements in ovariectomized +/+ and W/W^v mice

	Genotype			
	+/+		W/W ^v	
	Vehicle	Steroids	Vehicle	Steroids
No. of mice	5	6	5	6
Wet wt of uterine horn (mg)	5.27 ± 0.61	19.98 ± 2.01**	5.66 ± 0.48	17.59 ± 1.10**
Total no. of mast cells/horn	2739 ± 730	7901 ± 748**	0	0
Total uterine histamine content (ng/horn)	9.49 ± 2.32	15.55 ± 1.09**	1.90 ± 0.27	2.73 ± 0.50
Epithelial cell height (µm)				
Surface	13.45 ± 2.57	24.44 ± 2.20**	13.16 ± 1.20	23.57 ± 4.72**
Glandular	11.94 ± 1.42	15.23 ± 1.02**	12.27 ± 2.80	16.23 ± 1.16*

Values are mean ± s.e.m.
* *P* < 0.05, ** *P* < 0.01 compared with corresponding vehicle-treated mice.

As shown in Table 1 the total uterine histamine content of +/+ mice increased significantly with steroid treatment, reflecting the large increase in mast cells per uterine horn. The pool of histamine within the uterus of W/W^v mice that is not associated with mast cells responded to steroid treatment with a slight but non-significant increase.

As an additional measure of uterine responsiveness to steroids, we examined the influence of steroids on endometrial surface and glandular cell heights in ovariectomized +/+ and W/W^v mice. Steroid treatment caused a similar significant increase in the cell heights of both epithelial cell populations (Table 1).

Discussion

The suitability of using +/+ and W/W^v mice to study the role(s) of mast cells or histamine (e.g. mast-cell histamine *versus* non-mast cell uterine histamine) in the process of implantation and the initiation and development of a decidual cell response is dependent on the ability of the uterus of W/W^v mice to respond to exogenous steroids after ovariectomy. This is necessitated by the fact that the ovaries of W/W^v mice lack ovarian follicles or corpora lutea and hence are acyclic. In addition, our studies show that W/W^v ovaries contain enlarged pale-staining stromal cells which at the ultra-structural level contain numerous mitochondria, abundant smooth endoplasmic reticulum and many lipid droplets. These cytological features are consistent with steroid synthesis but the specific product and amount is not known. Shin, Hendrickson & Murphy (1972) have reported that these apparently hyperplastic stromal cells contain the enzyme 3β-hydroxysteroid dehydrogenase. Murphy & Beamer (1973) have shown that, in spite of an absence of follicles and corpora lutea, the vagina and endometrium of W/W^v mice were not atrophied like those of an ovariectomized female. Results of this type dictate the use of ovariectomized W/W^v mice since the ovaries seem capable of chronic, low level steroidal stimulation of unknown identity.

The results of our ovariectomy experiments indicated that the uteri of ovariectomized W/W^v and +/+ mice are able to respond similarly to exogenous steroid hormones. In comparison to non-ovariectomized mice, ovariectomy caused a decrease in uterine horn wet weight in +/+ and W/W^v mice. Likewise, ovariectomy caused a decrease in endometrial surface and glandular epithelial cell heights in mice of both genotypes while steroid treatment increased cell heights equally.

PLATE 1

Fig. 1. Light micrograph of a paraffin-wax section of ovary (O) from a mast cell-deficient mouse. Note the total absence of follicles and distinct corpora lutea. The ovarian stroma consists of large, pale-staining cells. H&E, $\times 350$.

Fig. 2. Higher magnification of Fig. 1. Note the large cells which form the majority of the ovarian stroma. These pale-staining cells have nuclei with distinct nucleoli. H&E, $\times 700$.

Fig. 3. Light micrograph of a 1.0 μ m plastic section. Note that the large ovarian stroma cells contain distinct lipid droplets (arrows) within their cytoplasm. Toluidine blue, $\times 890$.

Fig. 4. High-magnification electron micrograph of an ovarian stromal cell of a mast cell-deficient mouse. Numerous membrane-bound lipid droplets (L), vesiculated mitochondria (M) and abundant profiles of smooth endoplasmic reticulum (arrows) are evident. $\times 30\,000$.

Our histological results, using serial uterine sections, indicate the total absence of mast cells within the endometrium and myometrium of W/W^v mice. These results are important since Hatanaka *et al.* (1982) used only random histological sections in failing to detect mast cells in the uteri of W/W^v mice. Our results are additional proof that the uterus of W/W^v mice is completely lacking in mast cells. Steroid treatment caused an increase in total uterine mast cells in +/+ mice but failed to elicit mast cell differentiation from precursors or migration of mast cells into the uterus of W/W^v mice. Since W/W^v mice respond identically with regard to steroid-induced changes in uterine wet weight and endometrial cell heights, these responses appear to be independent of the presence of mast cells.

Because histamine is present in the uterus of ovariectomized vehicle-treated W/W^v mice and steroid treatment causes an increase in this level, in the absence of mast cells, a non-mast cell pool of histamine exists in the uterus of W/W^v mice which is responsive to steroids. The location of this histamine pool is not known, but may be associated with endothelial cells (Robinson-White & Beaven, 1982). The existence of a non-mast cell pool of histamine in other tissues has been suggested by biochemical (Beaven, Horakova, Seners & Brodie, 1968; Huszti, Kenessy, Kurti & Sourkes, 1977; Johnson, Ellis & Mitoma, 1973) and histochemical (El-Ackad & Brody, 1975) studies. Yamatodani, Maeyama, Watanabe, Wada & Kitamura (1982) demonstrated the presence of non-mast cell histamine in the brain, stomach, liver, kidney, spleen and skin of W/W^v mice. The significance of the uterine pool of non-mast cell histamine with regard to elicitation of a decidual cell response and/or implantation is yet to be determined. For example, the weight increase and oedematous changes that are associated with steroid treatment in W/W^v mice may be dependent to a certain degree on this pool of histamine.

Since W/W^v and +/+ mouse uteri respond to steroids in a similar manner, our results verify the feasibility of using these animals to investigate the role of uterine mast cells and non-mast cell uterine histamine in the process of implantation and the decidual cell response. By comparing the response of W/W^v mice after various treatments, we can determine whether uterine mast cells and/or non-mast cell uterine histamine are required for the response. Hatanaka *et al.* (1982) produced a decidual cell response in W/W^v mice using trauma as the stimulus. However, since trauma induction of a decidual cell response is only progesterone-dependent (Finn & Hinchliffe, 1964) there is still question as to whether mast cells are involved in the physiological induction of a decidual cell response or implantation which requires both oestrogen and progesterone. It is possible that the small amount of non-mast cell uterine histamine present in W/W^v mice is involved in a trauma-induced decidual cell response but would be insufficient in conjunction with a more physiological inducer (e.g. intrauterine oil injection) (Finn & Hinchliffe, 1964). Alternatively, the decidual cell response may be dependent on other cell populations which are not deficient in W/W^v mice.

Our results demonstrate the feasibility of using ovariectomized, steroid-treated W/W^v mice and their congenic mast cell-normal littermates in the study of the role(s) of the various histamine pools on implantation and the decidual cell response.

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