Research Articles

A study of granulated metrial gland cell differentiation in pregnant, macrophage-deficient, osteopetrotic (*op/op*) mice

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Abstract. A population of uterine natural killer (NK) cells, commonly called granulated metrial gland (GMG) cells, differentiates in the mouse uterus during normal pregnancy. Little is known regarding the process of differentiation of GMG cells or of other NK cell subsets. It has been suggested that macrophage precursors, under the combined influences of the cytokine growth factors colony stimulating factor-1 (CSF-1) and interleukin-2, become NK-cell like in morphology, pattern of target cell lysis and surface antigen phenotype. Mice expressing the mutation osteopetrosis (*op/op*) are unable to produce the cytokine CSF-1. To determine whether CSF-1 is required for the successful differentiation of uterine NK cells, implantation sites in pregnant, *op/op* mice were studied histologically. GMG cell differentiation appeared to progress normally in *op/op* mice studied between days 7 and 14 of gestation. Thus, the growth factor CSF-1 is not required for differentiation of the uterine NK cell subset known as GMG cells and probably GMG cells do not differentiate from macrophage precursor cells which are deficient in *op/op* mice.
Key words. Uterine NK cells; murine pregnancy; colony-stimulating factor-1 (CSF-1); NK cell differentiation; osteopetrosis.

Significant numbers of granulated metrial gland (GMG) cells are found in the murine uterus only during pregnancy[1,2]. Precursors of GMG cells appear to be a resident uterine cell population that responds to hormonal factors[1,3,4]. GMG cells are localized to the mesometrial triangle and decidua basalis of each implantation site by day 7 of gestation[5]. They proliferate in these sites for 2–4 days, achieving 15–20% of the tissue composition, and then become post mitotic. GMG cells appear to migrate throughout normal placentae, being found within blood vessels and labyrinthine sinusoids and they display significant motility in culture[6]. By day 15 of gestation degenerative changes are apparent within the GMG cell population and by term they are rare within the uterus.

GMG cells are bone marrow derived cells[7] that have a large, granulated lymphoid morphology, similar to that which has been described for natural killer (NK) lymphocytes. Recent experimental studies have established that GMG cells must be a subset of NK cells since they share the phenotypic markers asialo-GM1[8], LGL-1[9], NK1.1[2], Thy-1[8], FcR[10] and CD45[2] and lack CD3[2], CD4[8] and IgM[8]. Moreover, GMG cells produce the lytic pore-forming proteins of lymphocytes (perforin and serine esterases[11,12]) and can be induced by interleukin-2 (IL-2) to transcribe interferon-gamma[13] and to lyse the NK cell target cell line YAC[12–14] but not the LAK-cell target cell line P815[12]. The differentiation process for the NK cell lineage remains somewhat vague and incompletely defined[15–17]. Recently it was suggested that macrophage precursor cells, under the combined influences of the macrophage growth factor colony stimulating factor-1 (CSF-1 or M-CSF) and IL-2 can differentiate to resemble NK cells[17,18]. Availability of the mouse mutant osteopetrosis (*op/op*) which has an absolute deficiency of CSF-1[19–21] attributable to an inactivating mutation within the CSF-1 gene[22], provides a tool for assessment of the requirement for CSF-1 during the differentiation of GMG cells. Osteopetrotic mice are somewhat infertile[23] and have a deficiency in macrophages[19,20]. Indeed, uterine macrophages cannot be detected in *op/op* mice except during early pregnancy and these cells are uncharacteristic in their morphology[23]. If GMG cells in the pregnant *op/op* uterus are abnormal in appearance or are infrequent, the hypothesis that GMG cells could differentiate from macrophage precursor cells would be supported. Alternatively, if GMG cells in the pregnant *op/op* uterus are normal in appearance and frequency, it would be unlikely that GMG cells differentiate from macrophage precursor cells under the influence of CSF-1. Thus, a morphological assessment of GMG cells was undertaken in pregnant *op/op* mice.

Materials and methods

Animals. Heterozygote +/*op* and homozygote *op/op* mice were purchased from the Jackson Laboratories (Bar Harbor, ME) and bred at the Albert Einstein College of Medicine[23]. At 10 days of age the *op/op* mice were distinguished from their +/+ and +/*op* littermates by the absence of incisors and by a domed skull. After weaning the mice were fed a diet of powdered chow and liquid baby formula (Enfamil, Bristol Myers). Female *op/op* mice were selected for estrus and paired to +/*op* males.

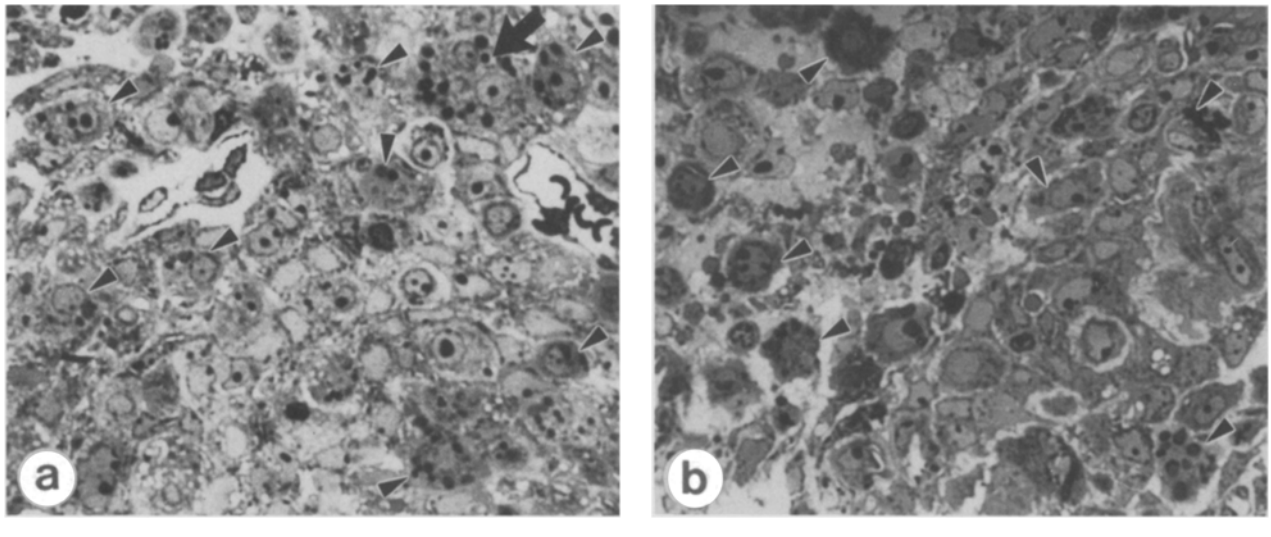
The morning of vaginal plug detection was designed as day 0 of pregnancy. Pregnant heterozygote, +/*op* females were used as controls. All mice were 8–12 weeks of age.

Tissue processing. Virgin (number of +/*op*:*op*/*op* = 1:2) and pregnant females from each of days 7 (2:1), 10 (1:3), 12 (2:2) and 14 (1:2) of gestation were sacrificed by cervical dislocation. Uteri were fixed in 2.5% glutaraldehyde – 2% paraformaldehyde in PBS (pH 7.4) or 3.7% paraformaldehyde in PBS (pH 7.2), and embedded in Epoxy resin or in paraffin. From Epoxy resin blocks 1-μm sections were prepared and stained with 1% toluidine blue. From paraffin blocks 5-μm sections were cut transversely through the center of the implantation sites and through interconceptual regions. Sections were stained using periodic acid-Schiff (PAS) reagent, with or without previous diastase digestion. Tissue samples were collected from at least 2 implantation sites and 2 interconceptual sites in each pregnant uterus.

Results and discussion

GMG cells were present in the uteri of *op*/*op* females on each of days 7, 10, 12 and 14 of gestation and resembled GMG cells found at the same times in +/*op* females (fig., a and b). The GMG cells identified in these mice are similar in morphology, site of localization and frequency to those reported in the literature for other randombred, inbred, wild and mutant mice[1, 5, 11, 24].

At day 7 of gestation, GMG cells containing diastase-resistant, PAS-positive granules were detected throughout the endometrium of implantation sites of *op*/*op* females, but more GMG cells were present on the mesometrial side than on the antimesometrial and the lateral sides. GMG cells were rarely observed in interconceptual regions. From days 10–14 of gestation in *op*/*op* mice, GMG cells possessing typical granules were localized to the decidua basalis and the metrial gland (fig., b) and had disappeared from both the antimesometrial and lateral decidua. During this period GMG cell numbers continued to increase in the metrial gland (table) and the size of the cells and of their granules also increased. Mitotic figures were readily identified among the GMG cells at day 10 of gestation. Binucleate GMG cells (fig., a) were sometimes observed particularly at day 10 when 7/407 (1.7%) *op*/*op* and 4/328 (1.2%) +/*op* GMG cells were binucleate. Binucleate cells are found among GMG cells from other mice[1]. GMG cells were detected in blood vessels of both the metrial gland and the decidua basalis of *op*/*op* and +/*op* mice and in intercellular spaces of the decidua basalis between days 10 and 14. Thus, no differences could be identified in the distribution, morphology or numbers of GMG cells between *op*/*op* and +/*op* mice. Since GMG cells differentiate in *op*/*op* mice, GMG cells do not require CSF-1 at any stage to induce a differentiation event. Further CSF-1 is not required for maintenance of GMG cell viability. CSF-1, although reported to have some chemotactic properties[25] does not play any role in the pattern of localization or traversing of blood vessels by GMG cells. Since the GMG cells found in *op*/*op* mice are normal in frequency and in appearance it is unlikely that GMG cell precursors are macrophage lineage cells, although this cannot be excluded absolutely since the cytokine granulocyte-macrophage-CSF (GM-CSF) is present in mice and is greatly elevated during their pregnancies[26]. GM-CSF can support the differentiation of cells from macrophage precursors[27]. An unusual population of small uterine macrophages has been defined by the antibody F4/80 in pregnant *op*/*op* mice[23]. These cells have a shorter life span than macrophages from the pregnant +/*op* uterus and are localized to the



Metrial gland from an +/*op* (a) and an *op*/*op* mouse (b) at day 12 of pregnancy. GMG cells (arrowheads) having typical granules are present. A binucleate GMG cell is observed (arrow). No significant morphological differences exist between these GMG cells. 1-μm section from epoxy resin embedded tissue stained with toluidine blue. ×640.

Frequency of granulated metrial gland cells per microscopic field[a] of metrial gland

Day of gestation	Genotype of female					
	op/op			*+/op*		
	No. of mice	No. of implantation sites	No. of GMG cells/400× field[b]	No. of mice	No. of implantation sites	No. of GMG cells/400× field
10	3	6	35,38,41,42,44,53	1	4	42,43,49,58
14	2	4	53,57,60,64	1	2	64,70

[a] Viewed at 400× magnification; [b] each number represents the result from one implantation site.

mesometrial triangle, the site of GMG cell development. Thus it remains possible that macrophages may influence the differentiation of GMG cells or that uterine macrophages may respond to the same differentiation signals as GMG cells.

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Mechanoelectrical transduction, ion movement and water stasis in uromodulin

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Abstract. Mechanical movement of a column of the urinary glycoprotein uromodulin modulates an applied voltage. This change is a property of the glycoprotein and its interaction with the walls of the container and is related to its capacitance. The voltage modulation is not accompanied by changes in rotationally restricted water as has been reported for hyaluronic acid. Diffusion experiments with tritiated water also support the hypothesis that uromodulin acts as a water barrier, but allows ion movement.
Key words. Uromodulin; glycoprotein; hyaluronic acid; electret; electrical transduction.