# A Study of Granulated Metrial Gland Cells in the Pregnant, Alymphoplasia (*aly*/*aly*) Mice

Yasuo KISO, Ken KUSAKABE, Yoshiko TOKUNAGA, Makiko MAKITA, Toshiya OKADA, and Fumihiko SASAKI

*Department of Veterinary Anatomy, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 593, Japan*

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ABSTRACT. During pregnancy, a population of uterine NK cells, commonly called granulated metrial gland (GMG) cells, differentiates in the uterus of both immune competent and various immunodeficient mice. Regulatory mechanisms controlling the differentiation of GMG cells are not fully known. It has been proven that GMG cells are derived from bone marrow, appear under the influences of progesterone and estrogen, do not require the presence of an embryo, and are associated in rodents with decidualization of the uterine stroma. Mice of genotype *aly*/*aly* are genetically deficient in lymph nodes and Peyer's patches due to a lymphoid-associated mesenchymal disorder. They are considered to be a useful model for the study of interactions between lymphocytes and stromal components. This immunodeficient animal is completely different from *nu*/*nu* and *scid*/*scid* mice who differentiate GMG cells during pregnancy. To determine whether the differentiation of GMG cells depends on mesenchymal interactions in the uterus, *aly*/*aly* mice were studied histologically between days 10 and 14 of pregnancy for differentiation of GMG cells and development of the metrial gland. Metrial gland tissue was present and appeared normal in *aly*/*aly* mice. There were no significant differences in the distribution of GMG cells in comparison to control pregnant *aly*/+ mice. Fewer GMG cells were present in *aly*/*aly* mice than *aly*/+ mice on days 12 and 14 of pregnancy. The features of individual GMG cells were different on days 10 and 12 of pregnancy. GMG cells in *aly*/*aly* mice were small in size and the granules were poorly developed. By day 14, however, GMG cells acquired a mature size and the granules appeared mature. It is likely that GMG cell differentiation was delayed in pregnant *aly*/*aly* mice, due to a mesenchymal disorder affecting metrial gland development in this animal.—
KEY WORDS: alymphoplasia, granulated metrial gland cell, immunodeficiency, mesenchymal disorder, uterine NK cell.

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Significant numbers of granulated metrial gland (GMG) cells are found in the murine uterus only during pregnancy [2, 23]. GMG cells are bone marrow derived cells and their precursors have been found in the uterus of infant mice at 2 weeks after birth [13]. In adult females, GMG cell precursors differentiate into large, heavily granulated, mature GMG cells, as a response to the signals associated with decidualization and pregnancy. GMG cells are localized to a developing tissue region known as the metrial gland and to the decidua basalis of each implantation site by day 7 of pregnancy [23]. Immature GMG cells found in the early stages of pregnancy are agranular or have a few, small granules. By day 10 of pregnancy, GMG cells are mature large cells (up to 50 $\mu$m in diameter) that possess numerous, electron-dense granules containing a lytic protein, perforin [21, 22, 26]. Granules have a solid core in mice, but not in rat. GMG cells proliferate in the metrial gland until about day 12 of pregnancy, degenerate slowly and disappear from these sites by term [7]. GMG cells share many phenotypic markers with NK cells, e.g., asialo-GM1, Ly49G2 (LGL-1), NK1.1 and Thy-1 [4, 18, 20, 24].

Little is known regarding the process of differentiation of GMG cells. Previous studies using various immunodeficient mice have revealed that GMG cell differentiation does not require T cells, B cells nor macrophages [13, 14]. In immunodeficient, alymphoplasia mice, C57BL/6J-*aly*/*aly*, genetic mutation has induced a generalized lack of lymph nodes and Peyer's patches [19]. The *aly*/*aly* mice maintain H-2 histoincompatible skin grafts for more than 80 days although the surface phenotype of their T cells (ratio of CD4/CD8 and CD3/TcR-$\alpha\beta$) is similar to that in *aly*/+ controls. In addition, the *aly*/*aly* mice have a deficiency in humoral immunity detected as very low serum levels of IgM, IgG and IgA. Nevertheless, *in vitro*, spleen cells from *aly*/*aly* mice proliferate in response to T cell mitogens (ConA, PHA), a B cell mitogen (LPS) and allogenic lymphocytes in a one-way MLA. These data suggest that the immunodeficiency observed in *aly*/*aly* mice results from an abnormality in the microenvironment of its various lymphoid tissues. The interaction between homing receptors on lymphocytes with ligands on endothelial cells has been suggested as a possible deficit [17, 19].

We previously reported that GMG cells interact closely with extracellular matrices, a major component of the stromal environment which affect GMG cell distribution, viability, morphological transformation and chemotaxis [2, 3, 16]. We wished to determine whether development of metrial gland and differentiation and distribution of GMG cells were altered in *aly*/*aly* mice.

## MATERIALS AND METHODS

Homozygote C57BL/6J (B6)-*aly*/*aly* and heterozygote B6-*aly*/+ mice were purchased from the Japan Clea (Osaka, Japan) and bred within a specific pathogen free (SPF) facility at our college. *Aly*/*aly* females at 10 weeks of age were selected for estrus and paired to +/+ males, and the morning of vaginal plug detection was designed as day 0 of pregnancy. Pregnant *aly*/+ females mated by +/+ males were used as controls. Pregnant females were sacrificed by

cervical dislocation on days 10 (number of *aly*/*aly*:*aly*/+ = 5:3), 12 (4:3) and 14 (5:3) of pregnancy. For light microscopy, uteri were fixed in Bouin's solution and embedded in paraffin. Paraffin sections (6 $\mu$m) were prepared transversely through the center of implantation sites and stained using periodic acid-Schiff (PAS) reagent, with or without previous diastase digestion. For electron microscopy, tissue samples from the metrial gland were cut into small pieces and prefixed in 2.5% glutaraldehyde-2% paraformaldehyde in PBS (pH7.4), postfixed in 1% osmic acid in PBS and embedded in epoxy resin. Tissue samples were collected from at least 2 implantation sites in each pregnant uterus. GMG cells containing PAS-positive granules were counted in at least three randomly selected fields using two sections from each implantation site with the Cosmozone-1SB system (Nikon Inc., Osaka, Japan). Fields for enumeration were limited to the metrial gland, not including the decidua basalis nor the myometrium. The average number of GMG cells per field was calculated to estimate the cell density (cells/mm$^2$) for each uterus. The ANOVA system was performed for statistical analysis using the StatView IV program. A probability of $<0.05$ was considered to be sigificant.

## RESULTS

Metrial glands were present in the uteri of *aly*/*aly* mice on each of days 10, 12 and 14 of pregnancy and were structurally similar to those in *aly*/+ mice at each time matched stage (Fig. 1). GMG cells in *aly*/*aly* mice contained diastase-resistant, PAS-positive granules (Fig. 2). These cells were found only in the metrial gland and the decidua basalis, not in the antimesometrial side of the uterus or in the interconceptual sites at all stages tested. This pattern of localization is the same as the pattern reported for immune competent mice. No statistically significant differences were noted in the density of GMG cells within the metrial gland between *aly*/*aly* and *aly*/+ mice on day 10 of pregnancy. Fewer GMG cells were present in *aly*/*aly* mice than *aly*/+ mice on each of days 12 and 14 of pregnancy although the pattern of increase in cell density was the same (Table 1).

Ultrastructural analysis of GMG cells on days 10 and 12 of pregnancy revealed that GMG cells from *aly*/*aly* mice were small in diameter (up to 20 $\mu$m) and that the granules were irregular in shape and small in size (maximum diameter 1.0 $\mu$m) and few in number. Moreover, the organelles of GMG cells from *aly*/*aly* mice on days 10 and 12 of pregnancy were poorly developed (Fig. 3). Ultrastructure of GMG cells from control *aly*/+ mice did not show such findings and is the same as that reported in normal mice. The morphological features of GMG cells from *aly*/*aly* mice

Table 1. Granulated metrial gland cells (cells/mm$^2$) in the metrial gland in pregnant, *aly*/*aly* and *aly*/+ mice

| Genotype | Day of gestation | | |
|---|---|---|---|
| | 10 | 12 | 14 |
| *aly*/*aly* | 514 ± 56.5 (5) | 646 ± 86.8* (4) | 647 ± 56.8* (5) |
| *aly*/+ | 529 ± 36.6 (3) | 877 ± 29.8 (3) | 880 ± 43.2 (3) |

Values are mean ± SE. Numbers of animals appear in parentheses.
* $P<0.05$, vs *aly*/+ mice.

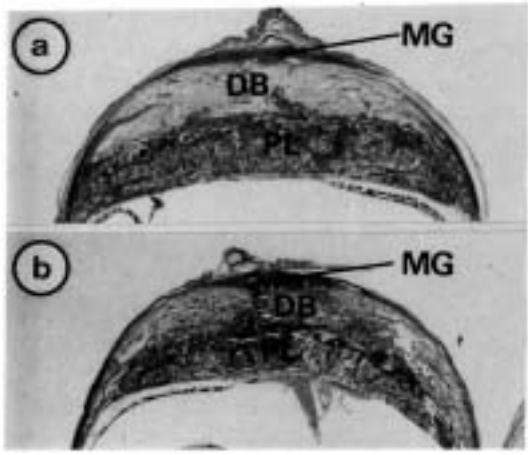


Fig. 1. Implantation site from the *aly*/*aly* (a) and *aly*/+ mouse (b) on day 12 of pregnancy. The metrial gland (MG) are present in both uteri. DB, decidua basalis; PL, placental labyrinth. PAS stain. × 9.

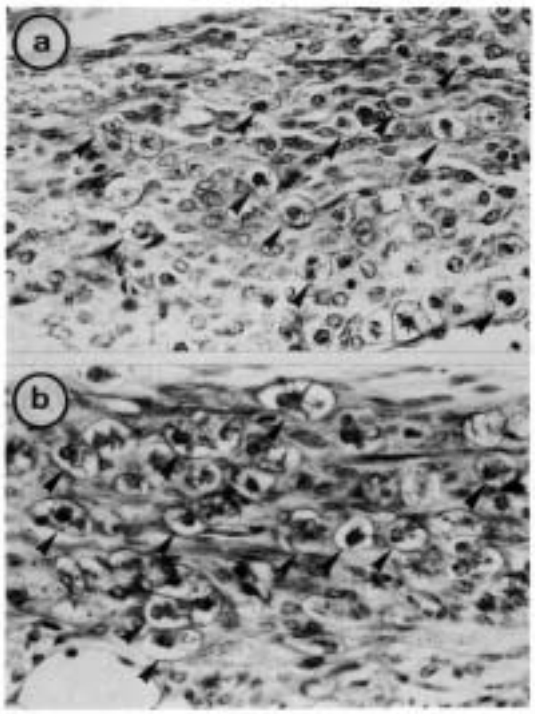


Fig. 2. Metrial gland from the *aly*/*aly* (a) and *aly*/+ mouse (b) on day 12 of pregnancy. GMG cells (arrowheads) with PAS-positive granules are seen in both metrial glands. GMG cells from the *aly*/*aly* mouse are smaller in size than those from *aly*/+ mouse. PAS stain. × 300.

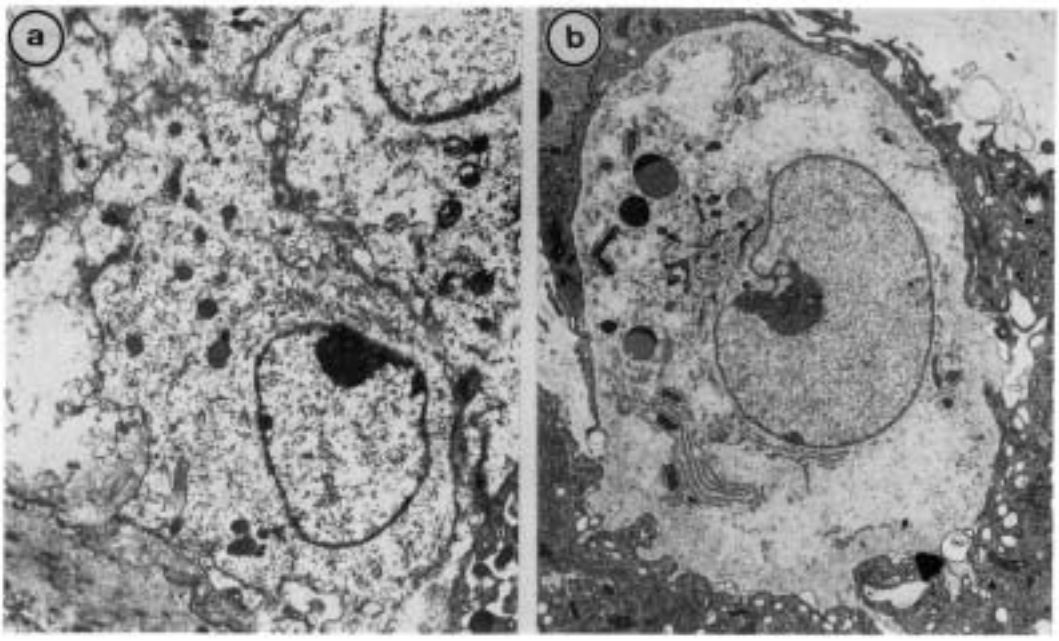


Fig. 3. GMG cell in the *aly*/*aly* (a) and *aly*/+ mouse (b) on day 10 of pregnancy. In contrast to the *aly*/+ mouse, GMG cell from the *aly*/*aly* mouse is small in diameter and the granules are small in size. Organelles of GMG cell from the *aly*/*aly* mouse are poorly developed. × 4,000.

resemble those seen in GMG cells on day 7 or 8 of pregnancy in immune competent mice and characterized the differentiating but not fully matured cell. However, by day 14, GMG cells from *aly*/*aly* mice increased in size (up to 40 $\mu$m in diameter) and the granules exhibited a mature appearance; i.e., regular round shape, large with a homogenous, electron-dense core in the central region and a membranous, cap-like structure in the peripheral region. Organelles such as mitochondria, Golgi complex and rough endoplasmic reticulum were well-developed (Fig. 4). Thus, in pregnant, *aly*/*aly* mice, GMG cells achieved maturity by day 14 of pregnancy.

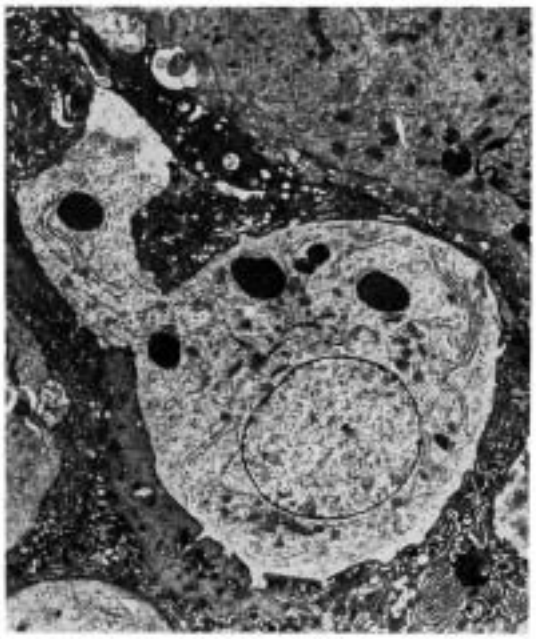

Fig. 4. GMG cell in the *aly*/*aly* mouse on day 14 of pregnancy. GMG cell has increased in size and the granules show a mature appearance. Organelles are well-developed. × 3,000.

## DISCUSSION

This study established that the metrial gland and GMG cells differentiated in pregnant, *aly*/*aly* mice, that GMG cells were fewer in number, and that GMG cell differentiation was delayed.

GMG cell differentiation does not require the presence of T cells, B-cells nor macrophages, since GMG cells were normal in pregnant, *nu*/*nu, scid*/*scid, RAG2-/-*, *mi*/*mi* and *op*/*op* mice [1, 9, 13, 14, 25]. In *aly*/*aly* mice, the interactions of homing receptors on lymphocytes with ligands on endothelial cells do not work because of the mesenchymal disorder of lymphoid tissues [17, 19]. The essential step in tissue-specific lymphocyte seeding is the tissue-specific attachment of circulating cells to the endothelial cells of target tissues via such interactions. GMG cells are uterine-specific lymphoid cells and GMG cell precursors, monoclonal antibody Ly49G2 (LGL-1) positive cells, appeared first in the uterus 2 weeks after birth, unlike any other lymphocytes [13]. GMG cells are thought to differentiate from their precursors resident in the uterus [15]. The interactions of homing receptors on GMG

cells or the precursors with ligands on endothelial cells in the metrial gland or the uterus could be distinct from those of T-cells and B-cells, since both T-cells and B-cells seeding are prenatal events in all the tissues including the uterus [5, 12]. Indeed, only the number of GMG cells in *aly*/*aly* mice was the same as that in *aly*/+ mice during early placentation. Alternatively, homing receptors on GMG cells might change in quality, as ECM receptors on GMG cells changed during implantation period [16]. To date extracellular matrices (ECMs) are known to be the factors that could influence the distribution, viability, transforming, chemotaxis and other behavior of GMG cells [2, 3, 16]. ECMs are a major component of the stroma, particularly in the pregnant uterus, and play a role in the interactions between endothelial or epithelial cells and mesenchyme [6, 10, 11]. These could account for poor development of GMG cells in *aly*/*aly* mice during early to mid-placentation. Thus, GMG cell differentiation may be delayed, due to a mesenchymal disorder affecting the metrial gland development in this animal.

Recently, the study using GMG-deficient, TgE26 mice indicated that GMG cells play critical roles in placental development, fetal survival and pregnancy success [8, 9]. Although fewer GMG cells were present in *aly*/*aly* mice than controls, *aly*/*aly* mice did not show fetal loss, placental reduction, nor abortion. The number of GMG cells on days 12 and 14 of pregnancy in *aly*/*aly* mice could be enough for placental growth and fetal survival, since that in TgE26 was approximately 3% of controls [9]. In TgE26 mice, fetal loss suddenly occurred between days 12 and 14 of pregnancy [8], when in *aly*/*aly* mice GMG cells acquired maturity. This accordance suggests that GMG cells have significant function during placentation period.

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