# Murine Granulated Metrial Gland Cell Population in Beige (*bg/bg*) and SCID (*scid/scid*) Genotypes

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(Received 24 August 1993/Accepted 25 November 1993)

ABSTRACT. Morphology of granulated metrial gland (GMG) cell, a uterine natural killer (NK) cell, was reported to be normal in pregnant uteri of NK cell-deficient mice and T-cell- and B-cell-deficient mice, but little is known about the number of GMG cells. To determine whether the number of GMG cells is influenced by such mutations, their number in the *bg* mice (genotype C57BL/6J-*bg/bg*) and SCID mice (genotype C.B-17/Icr-*scid/scid*) was compared to that of control mice on day 12 of gestation. GMG cells in these mutant mice were normal in number. Thus, the present results support the previous reports that GMG cells can differentiate normally in *bg* mice and that the GMG cell differentiation is not affected by functional T- nor B-cells.—KEY WORDS: beige mouse, granulated metrial gland cell, SCID mouse.

*J. Vet. Med. Sci.* 56(2): 415–416, 1994

Significant numbers of granulated metrial gland (GMG) cells are found in the murine uterus only during pregnancy [12]. GMG cells are observed in the mesometrial triangle and decidua basalis of each implantation site by day 7 of gestation, proliferate in the metrial gland by day 15 of gestation and degenerate during late gestation [12, 15]. GMG cells migrate throughout the placenta. Recent studies have established that GMG cells share the same lineage of natural killer (NK) cells [5, 9, 11, 18]. GMG cells show normal morphology in various mutant mice including beige (*bg/bg*), and severe combined immunodeficient (SCID) (*scid/scid*), as well as bone-marrow adoptively transferred mice [2, 3, 8, 12, 13, 17].

Beige mice have a deficiency of NK cell activity and SCID mice lack T and B cell functions [10]. Such a deficiency does not influence the morphology of GMG cells, but may affect the number of GMG cells within the metrial gland, which remains to be established in such mutant mice. To resolve this question, pregnant uteri were collected for enumeration of GMG cells from five groups of mice: conventional C57BL/6J (CO-B6) mice, specific pathogen free (SPF)-B6 mice, SPF-B6-*bg*/+ mice, SPF-B6-*bg/bg* (beige) mice, and SPF-C.B-17/Icr-*scid/scid* (SCID) mice.

All mice used in the present study were purchased from the Japan Clea (Osaka, Japan) and the Jackson Laboratories (Maine, U.S.A.). SPF mice were bred and housed within the barrier containment facility at our college and CO-B6 mice were housed in open standard cages in another building. Female mice were selected for estrus and paired to males of the same strain. The morning of vaginal plug detection was called day 1 of pregnancy. Mated females were sacrificed by cervical dislocation on day 12 of pregnancy. Uteri were fixed in Bouin's solution. Paraffin sections were prepared transversely at 6 $\mu$m through the center of implantation sites. Sections were stained using periodic acid-Schiff (PAS) reagent, with or without previous diastase digestion. Uteri from 2–5 females were examined for each group and tissue samples were collected from at least two implantation sites in each uterus. GMG cells containing PAS-positive granules were counted in at least five randomly selected fields in one to five 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 number of animals used per group is indicated in the table. Student's *t*-test was performed for statistical analysis.

GMG cells were easily identified because of their size and the presence of diastase-resistant, PAS-positive granules (Fig. 1). The data presented in Table 1 summarized the number of GMG cells in the metrial gland of each mouse strain on day 12 of gestation. There was no difference in the number of GMG cells between the CO-B6 and SPF-B6 mice, which suggests that the microbiological environment does not influence the number of GMG cells in at least the metrial gland. The insignificant difference in the number of GMG cells between the beige and SPF-B6 mice supports the previous histological observation that GMG cells differentiate normally in beige mice [2, 3, 17]. The insignificant difference in the number of GMG cells between the SCID and CO-B6 mice suggests that the number of GMG cells is not affected by the absence of functional T- and B-lymphocytes.

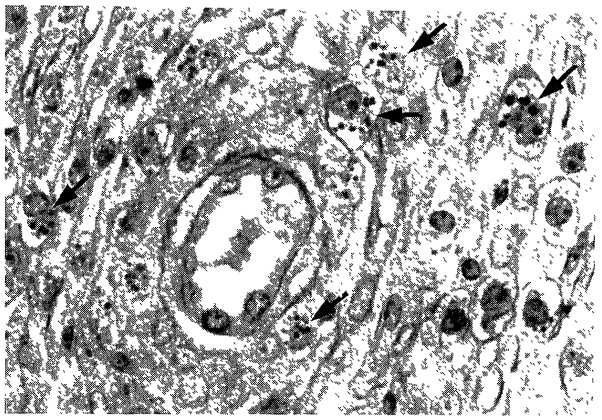

Fig. 1. Granulated metrial gland cells (arrows) with numerous PAS-positive granules in the metrial gland of C57BL/6J mouse on day 12 pregnancy. PAS. × 600.

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Table 1. Granulated metrial gland cells (cells/mm$^2$) in the metrial gland on day 12 of gestation

| | Normal females | | | Mutant females | |
|---|---|---|---|---|---|
| Genotype | CO-B6 +/+ | SPF-B6 +/+ | SPF-B6 *bg*/+ | SPF-B6 *bg/bg* | C.B-17 *scid/scid* |
| | 1002.94 ±48.79 (5) | 844.23 ±35.59 (5) | 907.77 ±38.24 (2) | 770.26 ±39.50 (4) | 1052.85 ±27.05 (5) |

Values are mean±SE.
Numbers of animals appear in parentheses.
Statistical comparisons between normal female groups are insignificant.
Statistical comparisons between the *bg/bg* and normal SPF-B6 mice are insignificant.
Statistical comparison between the SCID and CO-B6 mice is insignificant.

This study clearly establishes that the number of GMG cells in the beige and SCID mice is the same as that in the normal mice. The previous reports at both the light- and electron-microscopic level revealed that the morphology of GMG cells was normal in such mutant mice [2, 3, 17]. Although beige mice have a deficiency of NK cell activity, NK cells were reported to be generated *in vitro* from beige mice [6, 14]. Thus, the normal number of GMG cells in beige mice does not exclude the hypothesis that GMG cells are members of the NK cell lineage. In SCID mice, GMG cell precursors, monoclonal antibody LGL-1-positive cell, were reported to be normal number in the mature uterus [7]. Thus, the present results support the previous report that neither T cells nor B cells play a role in the differentiation of GMG cells [3, 7].

The functions of GMG cells during pregnancy remain to be fully defined. Only the similarities of GMG cells in the frequency and site of localization among mice including mutant mice suggest that they have functional significance during pregnancy. The differentiation of uterine NK/GMG cells is not equivalent to enhancement of cytotoxic activity [5]. On the other hand, GMG cells are known to secrete several cytokines including leukemia inhibitory factor [4] which is absolutely required for blastocyst implantation [16]. It is of great interest to determine whether GMG cells are secretory NK cells. The morphology of large, granular GMG cells suggests them to be a secretory type of NK cells. An electron-microscopic study using serial sections suggests that GMG granules are similar to those in secretory NK cells on morphological criteria (1, Kiso *et al.*, unpublished data). Further studies are needed to determine the function of GMG cells.

ACKNOWLEDGEMENT. This study was supported by a grant (No. 05660347) from the Ministry of Education, Sciences and Culture of Japan.

REFERENCES

1. Burkhardt, J. K., Hester, S., Lapham, C. K., and Argon, Y. 1990. *J. Cell Biol.* 111: 2327–2340.
2. Croy, B. A. and Kiso, Y. 1993. *Microsc. Res. Tech.* 25: 189–200.
3. Croy, B. A., Chapeau, C., Reed, N., Stewart, I. J., and Peel, S. 1991. pp. 168–188. *In*: Molecular and Cellular Immunology of the Maternal Fetal Interface (Wegmann, T. G., Gill, I. J., and Nisbet-Brown, E. eds.), Oxford University, Press, New York.
4. Croy, B. A., Guilbert, I. J., Browne, M. A., Gough, N. M., Stinchcomb, D. T., Reed, N., and Wegmann, T. G. 1991. *J. Reprod. Immunol.* 19: 149–166.
5. Croy, B. A., Reed, N., Malashenko, B.-A., Kim, K., and Kwon, B. S. 1991. *Cell. Immunol.* 133: 116–126.
6. Gunji, Y., Vujanovic, N. L., Hiserodt, J. C., Herberman, R. B., and Gorelik, E. 1989. *J. Immunol.* 142: 1748–1754.
7. Kiso, Y., McBey, B. -A., Mason, L., and Croy, B. A. 1992. *Biol. Reprod.* 47: 227–232.
8. Kiso, Y., Pollard, J. W., and Croy, B. A. 1992. *Experientia* 48: 973–975.
9. Linnemeyer, P. A. and Pollack, S. B. 1991. *J. Immunol.* 147: 2530–2535.
10. Lyon, M. F. and Searle, A. G. 1989. Genetic Variants and Strains of the Laboratory Mouse, 2nd ed., Oxford University Press, New York.
11. Parr, E. L., Parr, M. B., Zheng, L. M., and Young, J. D. E. 1991. *Biol. Reprod.* 44: 834–841.
12. Peel, S. 1989. *Embryol. Anat. Cell Biol.* 115: 1–112.
13. Peel, S. and Stewart, I. J. 1983. *Cell Tissue Res.* 233: 647–656.
14. Roder, J. C. 1979. *J. Immunol.* 123: 2168–2173.
15. Stewart, I. and Peel, S. 1978. *Cell Tissue Res.* 187: 167–179.
16. Stewart, C. L., Kasper, P., Brunet, L. J., Bhatt, H., Gadi, I., Kontgen, F., and Abbondanzo, S. J. 1992. *Nature (Lond.)* 359: 76–79.
17. Yamashiro, S., Bast, T., Kiso, Y., and Croy, B. A. 1991. *Proc. Xth Int. Symp. Morphol. Sci.* p. 165.
18. Zheng, L. M., Ojcius, D. M., Liu, C. C., Kramer, M. D., Simon, M. M., Parr, E. L., and Young, J. D. E. 1991. *FASEB J.* 5: 79–85.