NOTE *Anatomy*

Differentiation of Uterine Natural Killer Cells in Pregnant SCID (*scid/scid*) Mice

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ABSTRACT. To determine whether functional T- and B-cells can affect differentiation and/or proliferation of uterine natural killer (uNK) cells, their numbers in SCID mice (genotype, C.B.-17/Icr-*scid/scid*) were compared with those of control mice (genotype, C.B.-17/Icr-+/+) on days 8, 12 and 16 of pregnancy. Using biotinylated-*Dolichos biflorus* agglutinin (DBA) lectin staining, uNK cells can be readily classified into 4 subtypes, I to IV, from immature to mature types. The number of uNK cells was significantly lower in the decidua basalis of SCID mice than in that of control mice on day 8 of pregnancy. Particularly, the number of uNK cells of immature subtype II was significantly lower in SCID mice than in the control mice. By day 12, however, the uNK cell number in the SCID mice reached the same level as that of the control mice. It is likely that uNK cell differentiation in SCID mice was delayed during the early placentation period due to a lack of functional T and B cells.

KEY WORDS: differentiation, SCID mouse, uterine NK cell.

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Significant numbers of uterine natural killer (uNK) cells are found in the murine uterus only during pregnancy [7, 10, 13, 15, 19]. The uNK cells are observed in the metrial gland (MG) and decidua basalis (DB) of each implantation site, proliferate in the MG by day 12 of pregnancy, differentiate in the DB by day15 and degenerate during late pregnancy [1, 9]. The uNK cells can produce several cytokines and growth factors, having crucial roles in pregnancy maintenance, particularly decidual health and modification of spiral arteries [12, 16]. Peripheral NK cells and splenic NK cells are known to be affected in their differentiation and/or proliferation by cytokines and growth factors derived from functional T- and B- cells [14]. Since uNK cells are members of the NK cell lineage, their differentiation and/or proliferation may also be affected by T- and B- cells. We previously reported that the morphology of uNK cells in severe combined immunodeficient (SCID) mice deficient in functional T and B cells was similar to control mice on day 12 of pregnancy [18] and that the appearance of uNK cell precursors in SCID mice after birth was delayed compared with that of normal mice [5]. However, differentiation and/or proliferation of uNK cells in SCID mice during successful pregnancy remains to be fully understood. We wished to determine whether differentiation and/or proliferation of uNK cells were altered by absence of functional T and B cells using SCID mice.

SCID mice (genotype, C.B-17/Icr-*scid/scid*) and control mice (C.B-17/Icr-+/+) obtained from CLEA Japan (Osaka, Japan) were used in this study. Studies were performed according to protocols for animal use approved by the Yamaguchi University Animal Experimental Guidelines. Both mice were housed within the barrier containment facility at our University. Female mice were selected for estrus and paired with control males, and the morning of vaginal plug detection was called day 1 of pregnancy. Mated females were sacrificed by cervical dislocation under tribromoethanol anesthesia on days 8, 12 and 16 of pregnancy. The number of mice tested in each group was three. For lectin histochemistry analysis using biotinylated-*Dolichos biflorus* agglutinin (DBA) lectin (Vector Laboratories, Burlingame, CA, U.S.A.), the uteri were fixed in 10% buffered formalin, and sections 4 μm thick were prepared transversely through the center of implantation sites. The uNK cells can be readily divided into 4 subtypes, from immature to mature types, by DBA staining [9]. Deparaffinized sections were rehydrated and treated with 0.3% hydrogen peroxide for 15 min. After washing with 10 mM phosphate buffered saline (PBS), the sections were incubated with 1% bovine serum albumin (BSA) in 10 mM PBS for 30 min and then biotinylated DBA lectin diluted 1:2,000 in 1% BSA. Then, they were reacted with Avidin-Biotin Complex solution (Vector Laboratories) for 30 min at room temperature, washed with PBS and visualized with 3,3'-diaminobenzidine tetrahydrochloride. Sections without DBA lectin were regarded as the negative control.

According to the criteria of V. A. Paffaro *et al* [9], the four subtypes of DBA-positive uNK cells are as follows; subtype I cells were agranular, small round cells with a diameter of 9 ± 3 μm showing a weak DBA-positive reaction on the cell membrane. Subtype II cells were granular, round-shaped cells with a diameter of 13 ± 2 μm showing a weak DBA-positive reaction on the cell membrane and granules. Subtype III cells were heavily granular, large cells with a diameter of 26 ± 7 μm showing a moderate DBA-positive reaction on the cell membrane and granules. Subtype

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IV cells were heavily granular, large and irregularly shaped cells with a diameter of 30 ± 4 μm showing a strong DBA-positive reaction on the cell membrane and granules.

DBA-positive uNK cells were counted at three randomly selected fields using three sections from each placenta. Fields for enumeration were limited to the DB on days 8, 12 and 16 of pregnancy, while the MG was tested on days 12 and 16 due to its incomplete formation on day 8. The average number of uNK cells per field was calculated to estimate the cell density (cells/mm^2) for each placenta. The Mann-Whitney *U*-test was performed for statistical analysis. A probability of <0.05 was considered to be significant.

There were no differences in the number of implantation sites and morphology of placentas between the SCID and control mice (data not shown). The cell density of uNK cells was significantly lower in the DB of the SCID mice than in the control mice on day 8 of pregnancy (Figs. 1 and 2). Particularly, the number of subtype II cells was significantly lower in the DB of the SCID mice than in the control mice at that time (Fig. 3). Excepting day 8 of pregnancy, there were no differences in the cell number and differentiation of uNK cells between the SCID mice and control mice (Figs. 1 and 3).

The present study clearly established that differentiation of uNK cells was delayed due to deficiency of functional T- and B-cells during the early placentation period. Subtype I and II cells are immature and not functional, and those immature cells differentiate into mature subtype III cells that can play an essential role in modification and reconstruction of spiral arteries; ultimately, functional subtype III cells change to Subtype IV cells undergoing apoptosis [9, 12, 16]. Although the number of subtype II cells was significantly lower in the SCID mice on day 8 of pregnancy, differentiation and/or proliferation of uNK cells in the SCID mice returned to the control level by day 12 of pregnancy. Such recovery may be due to compensatory mechanisms related to differentiation and/or proliferation of uNK cells. It is well known that differentiation, proliferation and activation of NK cells are promoted by IL-2 derived from T cells and IL-12 derived from B cells [14]. Since uNK cells are members of the NK cell lineage, their differentiation may be delayed due to lack of their cytokines, particularly during early placentation period. However, since uNK cells themselves can produce IL-2 and since differentiation and proliferation of uNK cells can be promoted by IL-15 derived from decidual cells [2, 11, 17], the number of uNK cells in SCID mice could reach the same level as the control mice at the mid- and late placentation periods (days 12 and 16 of pregnancy). Otherwise, differentiation of uNK cells can be affected in an autocrine manner by epidermal growth factor [6] and by insulin-like growth factor derived from decidual cells as well as IL-15 [8]. It is possible that those cytokines and growth factors compensated uNK cells for differentiation and/or proliferation. Furthermore, chemokines may account for the normal uNK cell numbers in the SCID mice at days 12–16 of pregnancy. It was reported that human decidual NK (CD56$^+$CD16$^-$) cells reacted to chemoattractants derived from murine decidual cells [3, 4]. Chemokines

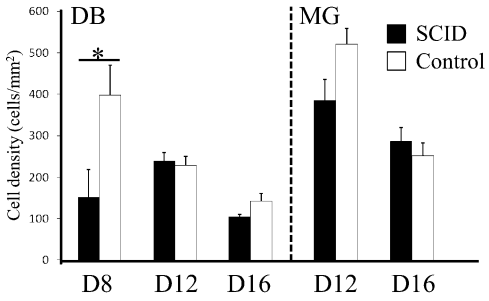

Fig. 1. The cell density (cells/mm^2) of uNK cells in the decidua basalis and metrial gland on the days 8, 12 and 16 of pregnancy. The cell density of uNK cells is significantly lower in the decidua basalis of the SCID mice than in the control mice on day 8 of pregnancy (* P<0.05). The number of mice tested in each group is 3. Histograms show the mean ± SE. DB: decidua basalis MG: metrial gland.

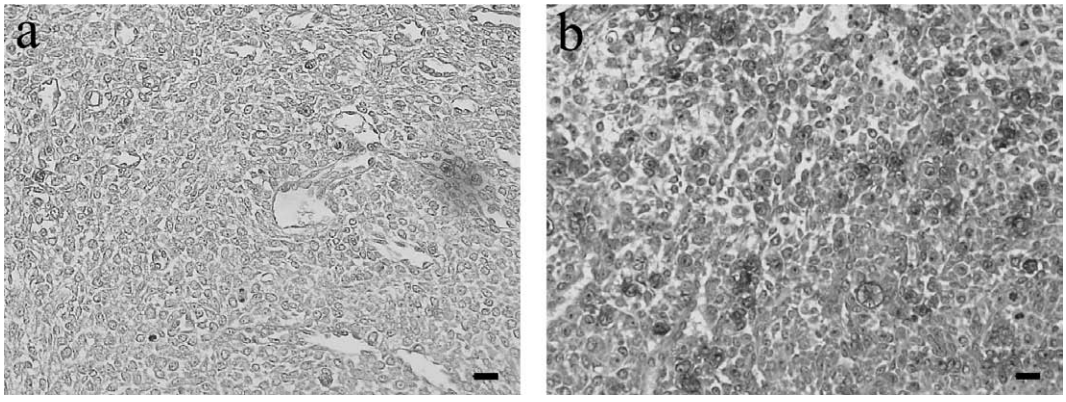

Fig. 2. Lectin histochemistry of uNK cells in the decidua basalis of SCID mice (a) and control mice (b) on day 8 of pregnancy. Subtype II cells in the control mice are widely distributed, while those in the SCID mice are distributed sparsely. DBA staining. Bar: 20 μm.

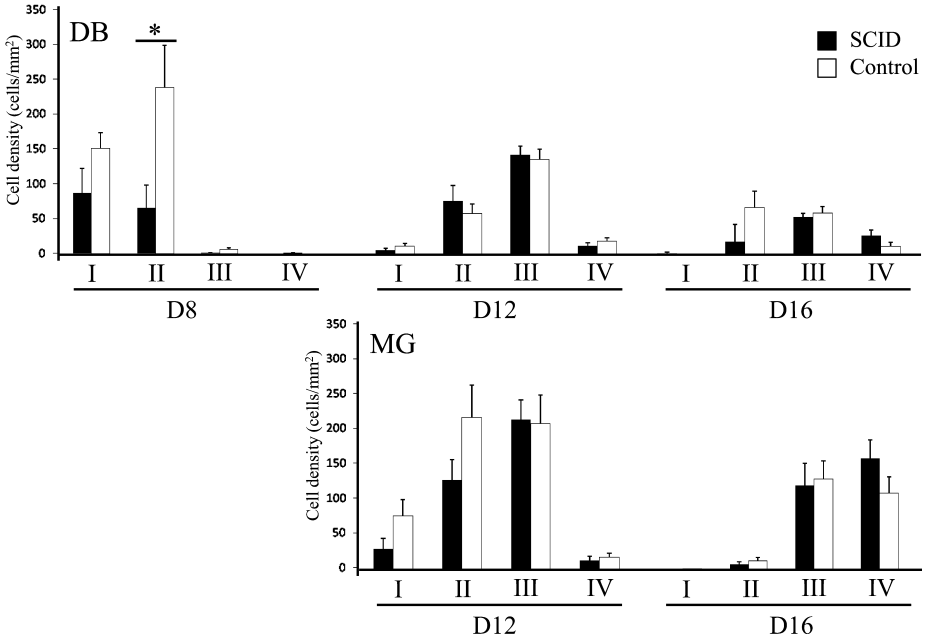

Fig. 3. The cell density (cells/mm^2) of each subtype of uNK cells in the decidua basalis and metrial gland on days 8, 12 and 16 of pregnancy. The cell density of subtype II cells is significantly lower in the decidua basalis of the SCID mice than in the control mice on day 8 of pregnancy (* P<0.05). The number of mice tested in each group is 3. Histograms show the mean ± SE. DB: decidua basalis MG: metrial gland.

such as CXCL-10 and CCL-8 derived from decidual cells may induce chemotaxis of uNK cells in such a way that the number of uNK cells in SCID mice might be similar to control mice at the mid- and late placentation periods. Further studies are needed to establish the relationships between uNK cells and T and B cells.

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