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Ultrastructural Study of Uterine Natural Killer Cells Found in Pregnant, Interleukin-2 Receptor β -chain Overexpressed Transgenic Mice

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Abstract. We previously reported that all fetuses died or were resorbed on day 12 of pregnancy (Day 1=the day of plug) in interleukin-2 (IL-2) receptor β -chain overexpressed transgenic (Tg2R β) mice. In this study, to clarify the role of uterine natural killer (uNK) cells in pregnancy, the ultrastructure of Tg2R β mouse uNK cells was analyzed using a transmission electron microscope. uNK cells and their granules on day 10 of pregnancy were larger in Tg2R β mice than control mice, indicating that differentiation of uNK cells in Tg2R β mice progressed rapidly. Additionally, the granules of uNK cells in Tg2R β mice on day 10 of pregnancy had an irregular morphology. The multivesicular regions were present in the cap structure of these granules, suggesting that the uNK cells of the Tg2R β mice had cytotoxic activity.

Key words: Interleukin-2 receptor β -chain overexpression, Transgenic mouse, Uterine natural killer (uNK) cell, Ultrastructure

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Murine uterine natural killer (uNK) cells dramatically increase in number and size from day 8 to 12 of pregnancy, and decrease from day 14 onward [1, 2]. The function of uNK cells remains to be fully defined. In tge26 mice deficient in uNK cells (T⁻/B⁺/NK⁻), female reproductive performance during pregnancy was quite poor. After reconstitution of uNK cells with bone marrow cells derived from *scid/scid* mice (T⁻/B⁻/NK⁺), fetal viability in tge26 mice recovered [3, 4], which clearly indicates that uNK cells play important roles in successful gestation. However, we cannot rule out the possibility that uNK cells can cause

abortion, since NK cells differentiate into lymphokine activated killer (LAK) cells through interleukin (IL)-2 stimulation, and since LAK cells can attack trophoblasts [5]. Indeed, uNK cells express IL-2 receptor, thus uNK cells could differentiate into LAK cells and have cytotoxicity for trophoblasts [6, 7]. We previously reported that severe abortion occurred in the IL-2 receptor β -chain overexpressed transgenic (Tg2R β) mice [8, 9]. In Tg2R β mice, all fetuses were lost on day 12 of pregnancy. The uNK cells of Tg2R β mice invaded into the labyrinthine zone. This invasion of uNK cells is not observed in normal mice. Therefore, the uNK cells of Tg2R β mice might have cytotoxicity for trophoblasts. In the present study, to clarify the role of uNK cells in pregnancy, the uNK cells of

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Fig. 1. Ultrastructure of uNK cells in NL - control mouse on day 12 of pregnancy. The uNK cells contain circular granules in the cytoplasm. These granules have a cap structure with a uniform electron density (arrow head). ($\times 5,000$) Inset: The cap structure (arrows) has a regular shape and uniform electron density. ($\times 7,000$)

Fig. 2. Ultrastructure of uNK cells in a Tg2R β mouse on day 10 of pregnancy. The cytoplasmic granules in the Tg2R β mouse are irregular in shape and have a cap structure that is also irregular in shape (arrow head). The cap structure in Tg2R β mice has a low electron density compared to NL. ($\times 5,000$) Inset: Multivesicular regions (arrows) are present in the cap structure of these granules. ($\times 7,000$)

Tg2R β mice were analyzed by transmission electron microscopy (TEM).

Materials and Methods

Mice

To obtain Tg2R β mice, C57BL/6 females were mated with Tg2R β males. Reverse transcription-polymerase chain reaction (RT-PCR) using mRNA from the peripheral blood was used to confirm whether or not transgenesis was successful in the next generation of mice (data not shown) [10]. The primers used were as follows: the sense primer was 5'-AATGTCTCTTGCATGTGGAG-3' and the antisense primer was 5'-AGACGCAGGAGAAGAAG-3'. Subsequently, female Tg2R β mice were mated with syngeneic males. Normal littermates (NL) without overexpression of IL-2 receptor β -chain were used as controls. All animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of Yamaguchi University.

Ultrastructural studies

For TEM observation, the decidua basalis of Tg2R β mice and NL (day 10 and 12) was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde/

0.1 M phosphate buffer (pH 7.4). Postfixation was performed in 1.5% osmium tetroxide in the same buffer. After dehydration with a graded series of ethanol and infiltration by propylene oxide, specimens were embedded in epoxy resin (Polysciences, Inc., Warrington, PA, USA). Ultrathin sections, 90 nm thick, were prepared and analyzed by TEM (H-7600L; Hitachi, Ibaraki, Japan).

Results

The uNK cells of Tg2R β mice and those of NL extended cytoplasmic processes that reflected on the ability of cells to migrate. Although uNK cell of NL contained small granules on day 10 of pregnancy, they had large and circular granules with a cap structure on day 12 of pregnancy (Fig. 1). However, on day 10 of pregnancy for Tg2R β mice, uNK cells and their granules were larger than those of NL on the corresponding day. The uNK cells on day 10 of pregnancy for Tg2R β mice were the same size as those on day 12 of pregnancy for NL, *i.e.*, approximately 35–40 μ m in diameter. The granules of Tg2R β mice on day 10 of pregnancy were irregular in shape, with a cap structure different from that in NL. The cap structure of Tg2R β mice

on day 10 of pregnancy contained multivesicular regions and was enlarged in comparison with that of NL. (Fig. 2).

Discussion

The size of the uNK cells of Tg2R β mice on day 10 of pregnancy were similar to those of NL on day 12 of pregnancy, suggesting that differentiation of uNK cells in Tg2R β mice progressed more rapidly than in NL. Such morphological changes could be caused by stimuli through the overexpressed IL-2 receptor β -chain. It is possible that not only IL-2, but also other cytokines, could be involved in the rapid differentiation of uNK cells in Tg2R β mice since pregnancy in IL-2 knockout mice is normally maintained and their uNK cells can normally differentiate [11, 12]. IL-15 is a candidate to accelerate differentiation of uNK cells in Tg2R β mice because of its high affinity to the IL-2 receptor β -chain [13, 14]. This is also supported by the report that human decidual NK cells analogous to murine uNK cells were activated by IL-15 [15]. The uNK cells of Tg2R β mice showed several different

features compared to NL, *i.e.*, enlargement of cytoplasmic granules with perforin and/or granzymes [16–19], and granules and a cap structure with an irregular shapes. The presence of the multi-vesicular region in the cap structure of cytoplasmic granules in the uNK cells of Tg2R β mice suggests, that according to morphological criteria, these were releasing type cells and that they had cytotoxic activity [20]. The uNK cells of Tg2R β mice might release perforin-enriched granules during mid-pregnancy earlier than NL, and perforin might be cytotoxic to trophoblastic giant cells, allowing uNK cells to invade the labyrinthine zone and ultimately cause abortion.

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