DIFFERENTIATION OF MURINE UTERINE NK CELLS

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Introduction

The cells known as uterine Natural Killer (uNK) cells are present in the uterus during normal pregnancy [1]. Murine uNK cells are characterized by the large granules stained by periodic acid Schiff (PAS), and are localized in the metrial gland and the decidua basalis of the placenta. The granules contain numerous enzymes, including granzymes , pore forming protein (perforin) and serine esterases, while uNK cells can produce several cytokines, including IL-1, CSF-1, LIF, EGF, and also INF-γ[2-5]. The uNK cells are recognized in the pregnant uterus of human, mouse, rat, and other species of mammals. The uNK cells are suggested to have significant function for maintenance of pregnancy through the placental vascular formation and modification[6,7]. Mechanism of uNK cell differentiation remains to be fully defined, although these cells are derived from the bone marrow[8,9]. The progenitors, that are small and agranular, increase in number and differentiate to large granular lymphocytes during the decidualization process. The uNK progenitors, Ly49G2$^+$ cells, are found in the uterus of infant mice at 2weeks after birth [10]. We wished to establish if the uterus is self sufficient in uNK progenitors or if replenishment of the lineage requires a progenitor influx from peripheral tissues. Also, we wished to establish if progesterone plays an essential role in differentiation of unk cells.

Methods and Materials

ICR mice were used as the recipient and the donor. Female mature, immature, and postnatal mice were used as donors to investigate the interaction between the uterus development and uNK cell differentiation. Female mature and immature mice were used as recipients. Mature and immature virgin mice as recipients were injected progesterone under the skin every 24 hours to investigate the relation between progesterone and uNK cell differentiation. Uterine tissue segments were collected from the donor, placed into the renal sub capsular space. Mature and pregnant mice were selected for estrus and paired to ICR males, and the morning of virginal plug detection was designed as day 0 of pregnancy (Day 0). The uterine grafts were transplanted at Day 0, and sampled

on Day 7. Revisualization of the grafts and differentiation of uNK cells within the grafts was investigated histological. Furthermore, involvement of sexual maturation in revisualization of the grafts and differentiation of uNK cells was investigated using the immature uterine tissue in the same way, i.e., transplantation was performed on Day 0, and the grafts were sampled on Day 7. In this experiment, 2 mg progesterone was injected under the skin from Day 0 to Day 6 every 24 hours. Influences of progesterone to the graft tissue were also assessed. For light microscopy, samples were fixed in Bouin's solution and embedded in paraffin. Paraffin sections (4 μm) were stained using periodic acid-Schiff (PAS) reagent.

Results and Discussion

Decidualization and differentiation of uNK cells were observed in grafts from mature and immature donors, but no change in grafts from postnatal. These changes were not associated with the sexual maturation of recipients (Table 1). However, when using the immature as recipients, decidualization and differentiation of uNK cells were not observed in the immature uterus under progesterone existence (Table 2). These results revealed that progesterone has indirect effects to differentiation of uNK cells through decidual cells, and that differentiation of uNK cells was closely related to the stage of uterus development. Progesterone is a key hormone to induce decidualization of endometrial stromal cells. Expression of progesterone receptor (PR) in the murine uterus and decidualization of endometrium are synchronized. In rodents, PR expression on the stroma of uterus is observed on Day 12-15 after birth[11], which supports our results.

Table 1. Decidualization and uNK cell differentiation in the uterine graft from 3 kinds of donors.

Donor	decidualization in graft	uNK in graft
mature	+	+
immature	+	+
postnatal	-	-

Three kinds of recipients: pregnant mature, non-pregnant mature with progesterone treatment and immature with progesterone treatment.

Table 2. Decidualization and uNK cell differentiation in the uterus of recipients themselves.

Recipient	decidualization in uterus	uNK in uterus
mature	+	+
immature	-	-

Three kinds of donors: mature, immature and postnatal.

uNK progenitors flow out from the bone marrow, arriving finally at the uterus, where uNK progenitors develop to uNK cells with pregnancy. Which factors excepting progesterone can affect uNK cell differentiation remains an experimental questionz. Since proliferation of uNK cells occurs within the uterus, the uterus has the capacity for tissue specific renewal of the uNK cells. IN graft experiments using the immnodeficient recipients, whose marrow had no ability to generate NK cells, no uNK cells could be found, suggesting that the peripheral tissues, not the uterus itself, provide uNK progenitors[6]. Progesterone is an important factor to differentiation of uNK cells, because differentiation and proliferation of uNK cells and progesterone from ovary are synchronized[12, 13], and because murine uNK cells express PR[14]. Alternatively, progesterone may induce decidualization rather than uNK cell differentiation. Further studies are required to define uNK cell differentiation.

Acknowledgements

This study was supported by a grant (No. 12306015 and 13027241) for Scientific Research A of the Ministry of Education, Science, Sports and Culture, Japan.

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