# A STRUCTURAL ANALYSIS OF MATERNAL BLOOD VESSELS IN THE PREGNANT UTERUS OF MICE DEFICIENT IN UTERINE NATURAL KILLER CELLS

Yasuharu NAMBA, Eiichi HONDO and Yasuo KISO

Departments of Veterinary Anatomy, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan

## Introduction

During mouse pregnancy, various lymphocytes appear in the uterus. The exact roles of these cells for pregnancy remain to be fully defined, whereas the timing of the appearance and/or disappearance is well defined[1]. Especially, a NK cell lineage among uterine lymphocytes shows time-dependent change in morphology during pregnancy[2]. Abnormalities of these cells should cause various abortion during murine pregnancy.

Murine uterine NK (uNK) cell without granules are recognized as immature cells expressing Ly49G2 antigen during preimplantation stage[3]. Once implantation occurs, these cells initiate differentiation, and the size of mesometrial gland gradually increases. The uNK cells reach maximum in number and size at the 12th day of pregnancy (12 D). uNK cells in mid-pregnancy is quite unique in morphology. The cytoplasm contains large granules with a cap structure. The uNK cell has low cytotoxicity against the trophoblast[4], although cytoplasmic granules include cytotoxic proteins such as perforin and granzyme[5]. From 14 D onward, the number of uNK cells decrease in the mesometrial gland and decidua basalis, and no uNK cell is detected at the term of pregnancy. Because the appearance and disappearance of uNK cells synchronize with placentation, uNK cells have been suggested to contribute to formation of the placenta.

Recently, transgenic and knockout mice are exploited to analyze the function of uNK cell. Especially, TgE26, CD3ε gene overexpressed-transgenic mice, lack T, NK and uNK cells, but B cells are normal and present. In TgE26, implantation normally occurs, but high rate of abortion is caused during mid-pregnancy. The placenta of TgE26 is smaller than normal mice[6]. The region of decidua contains fewer decidual cells than normal mice. The blood vessels (BVs) in decidua, the spiral arteries, are abnormal. Small placenta of TgE26 should be due to the failure of the blood supply. The spiral arteries have the stenosed lumen and the thickened wall. But it is reported that transplantation of the bone marrow of SCID mice into TgE26 reconstitutes only uNK cells, avoids abnormality of spiral arteries and of decidua, and induces normal pregnancy[6]. This report suggests that uNK cells should

contribute to vasculogenesis. In this study, we discuss the relationship between uNK cells and vasculogenesis in the uterus using several abortion models.

## Materials and Methods

*Mice*

C57BL/6 (B6, control) were purchased from CLEA Japan Inc. TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ mice were kindly provided by Dr. A Croy (Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph). RAG$^{-/-}$γc-chain$^{-/-}$ is a double knockout mice lacking of recombinase activating gene (RAG)-2 and common cytokine receptor chain γ, common to IL-2, 4, 7, 9, and 15 receptors. All lymphocytes including uNK cells are lost in RAG$^{-/-}$γc-chain$^{-/-}$. B6, TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ were mated with allogenic males. The day, when plug was confirmed, is defined as 0 D. Uterus of each mouse was collected at 12 D.

*Light microscopy*

Uterus of each mouse were fixed with Bouin's solution by perfusion, and embedded in paraffin wax by the conventional procedures. Sections of 5mm thickness were prepared and stained with PAS reagent (Wako Pure Chemical Industries, Ltd. Japan.).

*Scanning electron microscopy*

Each mouse was perfused with phosphate buffered formalin, and a Mercox (Dainippon Ink and Chemicals, Inc. Japan.) resin was infused into abdominal aorta. The tissue of uterus were dissolved with 4% KOH. Then, the corrosion cast of maternal BVs in the uterus was obtained.

*Transmission electron microscopy*

The decidua basalis of B6, TgE26, and RAG$^{-/-}$γc-chain$^{-/-}$ was fixed in 2.5 % glutaraldehyde and 2 % paraformaldehyde/0.01 M phosphate buffer (pH 7.4). Postfixation was performed in 1.5% osmium tetroxide/the same buffer. After dehydration with a graded series of ethanol and infiltration by propylene oxide, specimens were embedded in epoxy resin. Ultrathin sections of 90 nm thickness were prepared and analyzed by TEM.

## Results and Discussion

For light microscopic analysis, the placenta of TgE26 on 12 D is smaller than controls. Placentae of RAG$^{-/-}$γc-chain$^{-/-}$ were the same size as controls. In the control, many of uNK cells concentrated around decidual arteries. However, no uNK cell was detected in the metrial gland and decidua of TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$. The walls of BV of TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ were thicker than the control, and the lumen of BV of TgE26 was significantly stenosed. On the other hand, the lumen of BV of RAG$^{-/-}$γc-chain$^{-/-}$ was middle enlargement between TgE26 and the control. The decidual cells of TgE26 were sparser than the control. In conclusion, vasculogenesis of uNK cell-

deficient mice was somewhat failed, and hypertension might locally occur. Because of the abnormality of vasculogenesis, blood supply to the fetal placenta may be disrupted. This coincides with the report that TgE26 offspring is smaller than the control[6].

The structure of BVs of TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ was largely similar to the control, but spiral arteries of these mice, derived from segmental artery and sending off radial artery, have stenosed lumen. Especially, spiral arteries of TgE26 indicated slightly spiral conformation, and some of them run straightly. As the structure of segmental and radial arteries of TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ is similar to the control, abnormality of BVs should be restricted to decidual arteries, i.e., spiral arteries. uNK cells could participate in the construction of spiral arteries by producing IFN-γ, because uNK cell produces IFN-γ and spiral arteries of IFN-γ$^{-/-}$ mice indicate imperfection of vasculogenesis [7].

By ultrastructural analysis, an abnormality of endothelial cells of TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ was detected. Many of endothelial cells were columnar cell and protruded into the lumen, and some of them contained compressed nuclei and cytoplasm with consistent electron density. Some of protruded endothelial cells of TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ were apoptotic or necrotic, and detached into the lumen. New endothelial cells emerged in the basal side of spiral arteries of pregnant RAG$^{-/-}$γc-chain$^{-/-}$. These cells were not observed in TgE26. The differences between TgE26 and RAG$^{-/-}$γc-chain$^{-/-}$ were limited in the regeneration of endothelial cells and the enlargement of the lumen of spiral arteries. Because it is suggested that IFN-γ is concerned with vasculogenesis of the spiral artery, these differences may result from difference of the amount of IFN-γ. The concentration of IFN-γ in the pregnant uterus of RAG-2$^{-/-}\gamma_c^{-/-}$ must be well defined. However, macrophages, neutrophils, and/or decidual cells should compensate for producing IFN-γ [8-11], if IFN-γ is essential for the vasculogenesis of murine pregnant uterus. Actually, the number of decidual cells of RAG-2$^{-/-}\gamma_c^{-/-}$ mice increased more than that of TgE26. Vasculogenesis of the spiral artery in RAG-2$^{-/-}\gamma_c^{-/-}$ mice might be induced by the supply of IFN-γ from abundant decidual cells. In conclusion, IFN-γ may involve in normal formation of spiral arteries and regeneration of endothelial cells.

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