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Extended Abstract
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Stem Cell Research 2018-Induction of Immune Tolerance towards Allogeneic Cells using Fetal Directed Placental Injection in a Murine Model- Yukiko Shimazu- Osaka University Graduate School of Medicine

Yukiko Shimazu[1], Masayuki Endo[1], Katsuto Tamai [2], Kei Takahashi[1] , Takekazu Miyoshi[3], Hiroshi Hosoda[4], Alan W. Flake[5], Tadashi Kimura[1] , Jun Yoshimatsu[3]

[1]Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Yamadaoka, Suita, Osaka, 565-0871, Japan; [2]Department of Stem Cell Therapy Science, Osaka University Graduate School of Medicine, Yamadaoka, Suita, Osaka, 565-0871, Japan; [3]Department of Perinatology and Gynecology, National Cerebral and Cardiovascular Center, Kishibe-Shinmachi, Suita, Osaka, 564-8565, Japan; [4]Department of Biochemistry, National Cerebral and Cardiovascular Center, Kishibe-Shinmachi, Suita, Osaka, 564-8565, Japan; [5]The Center for Fetal Research, The Children's Hospital of Philadelphia, Philadelphia, PA

Introduction

Fetal therapy is important in preventing lethal disease and also for diseases that can cause permanent organ damage before birth. If postnatal treatments are limited to palliative options, fetal therapy including intrauterine stem cell therapy may be offered . When allogeneic transplantation is performed in adults, the vigorous immune response against the transplant becomes the major problem. Intensive immunosuppression and myeloablation is required to prevent rejection or graft versus host disease. Therefore, immunologic tolerance is an important issue for stem cell transplantation success. Immunologic tolerance is defined as unresponsiveness to an antigen that is induced by previous exposure to that antigen. In utero, the immune system is immature . The fetus has been shown to accept allogeneic antigens, which introduces immune tolerance. During this period, stem cell transplantation is expected to result in successful engraftment without the need for myeloablation or immunosuppression. This reduces the risk of rejection reaction and myelosuppression. There have been previous studies, which have used in utero-transplantation in animal models [1,8,9]. Many of the animal disease models were successful in achieving a clinically significant level of chimerism [10]. In humans, stem cell transplantation in the fetus has been attempted for a variety of diseases . However, in utero bone marrow cell transplantation has only been successful in immune deficiency states, where donor cells have a competitive advantage. Recently, mesenchymal stem cell transplantation for osteogenesis imperfecta has resulted in a degree of clinical efficacy. However, these cases required both prenatal and postnatal transplantation . Despite successful results from fetal transplantation in animal models, results for human fetus transplantation have been limited. The development of the immune system is different in mice and humans, so the timing of when to induce immune tolerance is also different. In humans, the window for tolerance induction is thought to be limited to the first trimester, ending approximately 14 weeks after gestation . Chorionic Villus Sampling (CVS) is widely utilized for prenatal diagnosis. It is performed at between 10 to 14 weeks of gestation. The technique used for CVS is an attractive approach for delivering cells and/or foreign antigens to the fetus since it may be possible to achieve fetal tolerance at this appropriate time. We previously reported an early gestational placental injection of donor cells carrying a foreign protein into a fetal murine model, which induced immune tolerance against the foreign protein [15]. In this study, we utilized the same procedure to induce immune tolerance with early gestational stem cell transplantation into the placenta in allogeneic murine models.

MATERIALS AND METHODS

Ethical statement

All procedures in this study were carried out in strict accordance with the guidelines for animal experimentation from the Animal Research Committee of Osaka University and that of the National Cerebral and Cardiovascular Center. The protocol was approved by the Animal Research Committee, Osaka University (Permit Number: 24-079-018), and National Cerebral and Cardiovascular Center (Permit Number:

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13018). All surgery was performed under anesthesia, and all efforts were made to minimize suffering where possible.

Mouse recipients and donors

Eleven-day old embryos from Balb/C mice were designated as recipients in the allogeneic models. We used surrogate mothers by removing the influence of postnatal exposure to maternal alloantibody in breast milk [16]. Donor cells were harvested from C57BL/6TgN (act-EGFP) OsbY01 mice (kindly provided by Dr. Okabe, Osaka University, Genome Information Research Center) that had been maintained in our breeding colonies. Injected mice were housed in the Laboratory Animal Facility at the National Cerebral and Cardiovascular Center Research Institute. The experimental protocols were performed with approval from the Institutional Animal Care and Use Committees of the National Cardiovascular Center Research Institute.

Preparation of donor BMCs

Adult GFP-positive BMCs (B6GFP-BMCs) were isolated from B6GFP mice by flushing the ilium, humerus, tibia, and femurs with Ca/Mg-free Phosphate-Buffered Saline (PBS) using a 26- gauge needle. After filtration through a 40μm mesh filter, B6GFP-BMCs were centrifuged at 440 xg for 5 minutes at room temperature. After the red blood cells were lysed with lysing buffer, the B6GFP-BMCs were counted and Suspended in PBS at a density of 8 × 107 cells/ml for injection.

CONCLUSION

In conclusion, we utilized a technique similar to CVS to transfer donor cells carrying allogeneic cells into the fetal side of the placenta in the mouse models. This approach proved sufficient for induction of immune tolerance against the allogeneic cells in a murine model.

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endo@gyne.med.osaka-u.ac.jp

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