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A specific immune tolerance toward offspring cells is to exist after the mother lymphocyte infusion

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ABSTRACT

Purpose: To examine immune tolerance between maternal lymphocytes and offspring tissue after a donor lymphocyte infusion.
Methods: Mouse models were established by mating female BALB/c mice with male C57BL mice. Splenic lymphocytes from donors of different genetic backgrounds were labeled with carboxyfluorescein succinimidyl ester (CFSE), and 1×10^7 of the labeled cells were intravenously injected into a recipient. At 6 h, 24 h, 72 h and 120 h after the infusion, mononuclear cells in recipient spleen, liver, thymus, lymph nodes, and peripheral blood were collected. CFSE+, CFSE-, CD3+, CD8+, CD4+, CD19+, NK1.1+, CD25+, and CD127+ lymphocytes in those samples were analyzed by flow cytometry. The distribution of donor T cells, B cells, NK cells, helper T cells, cytotoxic T cells, and recipient regulatory T cells in the tissues were then analyzed.
Results: Maternal lymphocytes were more likely to survive in offspring. At 120 h after infusion, the percentages of maternal cells in the offspring were $0.52 \pm 0.11\%$ in lymph nodes, $0.97 \pm 0.04\%$ in peripheral blood, and $0.97 \pm 0.11\%$ in the spleen. Few donor cells, if any, were detected in these tissues at 120 h after aunt to child, father to child, and unrelated allogeneic infusions were performed. The subtype proportion of donor lymphocytes changed significantly in the recipient tissues. Recipient Treg cells increased in the mother to child group, but not in the aunt to child, father to child, and unrelated allogeneic groups, suggesting a decreased cellular immune response to allogeneic cells in the mother to child group. At 120 h after the infusion, no donor cells were detected in the recipient livers and thymuses of all groups, implying that donor cells were barely able to colonize in the liver and thymus.
Conclusion: Specific immune tolerance to maternal lymphocytes exists in offspring. An infusion of maternal donor lymphocytes may produce a relatively persistent effect of adoptive immunotherapy with reduced side-effects.

Adoptive lymphocyte infusion (ALI) has become an effective approach for treating leukemia and tumors (Maus et al., 2014; Collins et al., 1997; El-Jurdi et al., 2013), And is usually performed by one of three methods: (1) a donor lymphocyte infusion after an allogeneic hematopoietic stem cell transplant, (2) a cytokine-induced killer (CIK) infusion, or (3) a synthetic tumor antigen-stimulated lymphocyte infusion. While the infused lymphocytes may kill tumor cells in the patient's body, graft-versus-host disease (GVHD) always occur in patients transfused with large quantities of allogeneic lymphocytes (Lewalle et al., 2003). Ideally, the ALI produces an adoptive immune response to tumor cells, but only a small or no graft-versus-host response. Recent studies have shown that the prognosis of patients who receive a hematopoietic stem cell transplant (Ichinohe et al., 2004; Matsuoka et al., 2006) or solid organ transplant (Opelz, 1999; Andrassy et al., 2003) from their mother is better than that of patients who receive such transplants from an unrelated haploidentical donor. A mouse experiment showed that 57% of H-2(b/b) offspring of H-2(b/d) mothers accepted allogeneic heart grafts from H-2(d/d) mice for >180 days, while similar transplants were all rejected by day 11 in control mice (Andrassy et al., 2003). Another study (Nijagal et al., 2012) showed that biliary atresia patients who received a maternal liver graft had a lower rate of graft failure when compared with such patients who received a paternal graft. The presence of maternal cells in offspring may induce tolerance to noninherited maternal antigens (NIMAs) (Nijagal et al., 2012). In mouse models of bone marrow transplanta-

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tion, an offspring-to-mother transplantation from a NIMA-exposed donor reduced the morbidity and mortality of rates of GVHD in an antigen-specific manner (Ichinohe et al., 2004; Matsuoka et al., 2006). Several clinical studies also reported that the success rate of transplantation was higher and the incidence of GVHD was lower among recipients who received a graft from their mother when compared with recipients who received a graft from an unrelated haploidentical donor (Ichinohe et al., 2004; Matsuoka et al., 2006; van Rood et al., 2002; Tamaki et al., 2001). However, the in vitro T cell reactivity of healthy individuals toward their parents and/or siblings expressing the NIMAs showed no evidence being influenced by a NIMA effect (van den Boogaardt et al., 2005; Dutta and Burlingham, 2011). Therefore, the chimeric NIMAs and the specific immune tolerance to NIMAs in the offspring require further study.

In a previous study, we successfully infused large doses of maternal peripheral lymphocytes to treat offspring which had EBV-associated lymphoproliferative disorders (Wang et al., 2010). After the infusions, the enlarged lymph nodes shrank and serum EBV virus titers decreased significantly without accompanying severe rejection responses, such as rash or high fever. However, maternal lymphocytes quickly disappeared in the recipients' peripheral blood, and the mechanisms underlying the therapeutic effect are not yet fully understood. In this study, we used a mouse model to examine the survival and distribution of infused maternal lymphocytes as well as the tolerance of offspring to the maternal lymphocytes.

1. Materials and methods

1.1. Mice

Male C57BL (H-2^b) mice (aged 2–3 months) were originally purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Female BALB/c (H-2^d) mice (aged 2–3 months) were obtained from Vital River Laboratory (Beijing, China). F1 mice were produced by mating male C57BL (H-2^b) mice with female BALB/c mice (Fig. 1). The F1 mice were nursed by their corresponding mothers, and weaned at 21 days after birth. All animal experiments were performed in accordance with experimental animal ethical standards, and the study protocol was approved by the Peking University Institutional Animal Care and Use Committee.

1.2. Lymphocyte infusion

The parent mice of female BALB/c (H-2^d) or male C57BL (H-2^b) mice were used as donors for lymphocyte infusion, and the F1 mice served as recipients (Fig. 1).

Each donor mouse was sacrificed by cervical dislocation; after which, its spleen was removed and gently pulverized under sterile conditions. Mononuclear cells in the spleen cell suspension were extracted with lymphocyte separation medium (Vannucchi et al., 2000). The extracted cells were then diluted to a concentration of 2×10^7 cells/mL, and labeled with the fluorescent dye, 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR, USA) (Nilsson et al., 2001). An aliquot of labeled cells (1×10^7 cells in 0.5 mL) was then intravenously infused into each recipient mouse via its lateral tail vein.

1.3. Distribution of donor lymphocytes as determined by flow cytometry

At 6 h, 12 h, 24 h, 72 h, and 120 h after infusion, the spleen, liver, thymus, and lymph nodes were removed from each recipient mouse and gently pulverized. Samples of peripheral blood were collected from an angular vein. Mononuclear cells in the cell suspension were extracted with lymphocyte separation medium. Cells

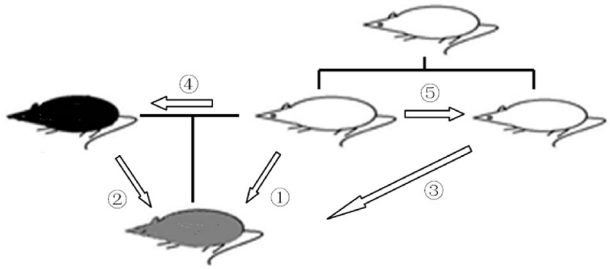

Fig. 1. CFSE labeled donor splenic lymphocytes were infused to recipients of different kinships.
Black is male C57BL(H-2^b) mouse; white are female BALB/c mice (H-2^d); the gray is offspring produced by mating C57Bl and BALB/c. The lymphocyte infusion relationship between donor and recipient: ① mother to child group; ② father to child group; ③ aunt to child group; ④ unrelated allogeneic group; ⑤ syngeneic group.

to be analyzed were diluted to a concentration of 10^6 cells/300 μL. CFSE-labeled donor cells were detected by using the FITC fluorescence channel of a flow cytometer (FACSCalibur, BD Biosciences; Franklin Lakes, NJ, USA). Unlabeled lymphocytes were used as negative control cells and labeled cells were used as positive cells to adjust the voltage, amplitude, and compensation settings of the flow cytometer, so that labeled and unlabeled lymphocytes could be accurately distinguished. The useful cell subgroups in SSC (ordinate)/FSC (abscissa) scatterplots were gated to exclude cell debris, and the cell subgroups were plotted as a histogram which had the cell number on the ordinate and CFSE intensity on the abscissa. The ratio of CFSE-positive cells to total cells represented the proportion of donor lymphocytes in tissue samples (Fig. 2). Except for when analyzing Treg cells, a minimum of 100,000 cells were analyzed per sample. When analyzing Treg cell populations (CD4+ CD25+ CD127-), at least 10,000 events were collected for each sample because of their low proportion, and this was especially true when counting donor Treg cells.

1.4. Subtypes of recipient lymphocytes as determined by flow cytometry

Rat anti-mouse CD3, CD8, CD4, CD19, NK1.1, CD25, and CD127 antibodies (Becton Dickinson BD, USA) were used to identify T cells, B cells, NK cells, and regulatory T cells (Treg), as well as CD4+ and CD8+ T cells by flow cytometry. Prior to identifying the lymphocyte subtypes, unlabeled and CFSE labeled cells were used to adjust the settings of the flow cytometer in order to accurately distinguish the donor and recipient lymphocytes.

1.5. Statistical analysis

Each experiment was performed in duplicate. Statistical analyses were performed using SPSS for Windows, Version 13.0. Chicago, IL: SPSS Inc. Numerical results are presented as the mean ± standard deviation (SD). Variables at different time points after an infusion were analyzed by ANOVA. Differences between two groups were analyzed by the independent-samples T Test. A P-value < 0.05 was considered statistically significant.

2. Results

2.1. Maternal lymphocytes were more likely to survive in lymph nodes, peripheral blood, and the spleen

The percentages of donor cells in lymph node tissue were highest in the mother to child and syngeneic groups. At 6 h, 24 h, 72 h, and 120 h after an infusion, the donor cell percentages in the