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UNRAVELING THE MECHANISM OF TOLERANCE INDUCTION FOLLOWING IN UTERO TRANSPLANTATION

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Aim of Study: In utero transplantation (IUT) of haematopoietic cells results in immune tolerance, allowing long-term haematopoietic engraftment and organ transplantation across immune barriers. In the present study we sought to determine the mechanism of tolerance induction following IUT of bone marrow (BM)-derived haematopoietic cells.

Methods: BM isolated from 6-week old B6GFP mice was sorted into four subpopulations based on c-kit and Sca1 expression after depletion of lineage-committed cell (Lin+). Allogenic IUT of selected subpopulations contained in 107 BM cells (positive control) was performed at E14 into Balb/c mice via the vitelline vein. Cells were tracked/phenotyped in the fetal liver and the immature thymus at E15-16. For assessment of tolerance each group received an additional transplant of 107 B6GFP BM cells at birth (P0). Haematopoietic engraftment of donor cells (% GFP+ within CD45+) was assessed at 4 weeks in blood. Statistical analysis was performed using 1-way ANOVA.

Main Results: Transplantation of 107 allogenic BM cells resulted in successful engraftment when injected at E14 (9.5±3.7%), but postnatal rejection when administered at P0 (0.01±0.01%, $p<0.001$). Lin- cells (105) engrafted in the fetal liver, differentiated into dendritic cells (DC; CD11c+) that homed to the thymus and induced tolerance (10.8±1.1%). This is in contrast to what we saw when IUT was performed with Lin+ cells (105), which did not induce tolerance (0.09±0.06%; $p<0.001$). Of the four Lin- subpopulations only cKit-/Sca1- cells generated DC that were found in the thymus at E16 resulting in tolerance (2.7x104 cells; 6.8±1.0; $p<0.01$ vs. other Lin- subpopulations and Lin+; $p>0.05$ vs. Lin-; Figure).

Conclusion: Tolerance induction post allogenic IUT depends upon Lin- c-kit- Sca1- cells, a minute sub-fraction of BM. Tolerance is mediated by timely thymic homing of fetal liver-generated, donor-derived, CD11c+ DC. This finding has important clinical implications for prenatal tolerance induction for cellular and organ transplantation.