KSN 2021
FULLY VIRTUAL MEETING
September 02 (Thu) - 05 (Sun)

Oral Communication Abstract
Presentation No. OC6-03 (Abstract Submission No. 2223)

Oral Communications 6 Sep. 3 (Fri), 10:40-12:40

Anti-C4d chimeric antigen receptor regulatory T cells suppressed allograft rejection in ABO-incompatible heart transplantation

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Objectives: Antibody-mediated rejection (ABMR) is the main hurdle in ABO blood group-incompatible (ABOi) transplantation. Recently, chimeric antigen receptor regulatory T cells (CAR Tregs) have been developed to improve antigen specificity, viability, and suppressive activity of Tregs. Interestingly, deposition of complement component 4d (C4d) is a marker of ABMR and is also found in most ABOi allograft tissues. Based on this finding, we developed anti-C4d CAR Tregs to suppress ABOi allograft rejection.

Methods: We generated anti-mouse C4d single chain variable fragment (scFv) using phage display and constructed anti-C4d CAR that consisted of extracellular anti-C4d scFv and intracellular CD28 and CD3ζ. Anti-C4d CAR Tregs were prepared by retroviral transduction of CAR into sorted $CD62L^+CD4^+CD25^+$ Tregs. Anti-C4d CAR Tregs were expanded by stimulation of anti-CD3/CD28 beads with rapamycin and IL-2. As a ABOi ABMR model, hearts from human blood group A-transgenic BALB/c mice were transplanted into C57BL/6J mice sensitized by A antigens. $CD45.1^+$ non-transduced, control CAR, or anti-C4d CAR Tregs were transferred into recipients.

Results:

Anti-C4d CAR Tregs expressed Foxp3, CD25, CTLA-4, LAP, and GITR to similar extent as non-transduced Tregs. Anti-C4d CAR Tregs specifically bound to C4d, were activated by binding to C4d, and their suppressive activity against *in vitro* T cell proliferation was similar as non-transduced Tregs. Next, adoptive transfer of anti-C4d CAR Tregs significantly prolonged mouse ABOi heart allograft survival than PBS control ($P < 0.05$), whereas non-transduced or control CAR Tregs did not. Antibody-mediated histologic injury and expression of IFN-γ and TNF-α in heart allografts were attenuated in the anti-C4d

CAR Treg group. Furthermore, infiltration of $CD45.1^+$ Tregs around $C4d^+$ endothelial cells in the anti-C4d CAR Treg group was more prominent than that in other groups.

Conclusions:

Anti-C4d CAR Tregs improved ABOi heart allograft survival by suppressing ABMR and are a promising therapeutic agent for controlling ABMR as well as ABOi allograft rejection.