DEVELOPMENT OF A METHODOLOGY FOR THE PRODUCTION OF SPECIFIC ALLOANTIGEN- REACTIVE HUMAN TREGS

Alaa Alzhrani, Joanna Hester and Fadi Issa

Transplantation Research & Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford

Background: Organ transplantation is limited by the need for life-long immunosuppression and its off-target side effects, which include life-threatening infection, malignancy and cardiovascular disease. Regulatory T cell (Treg) therapy has the potential to reduce the need for immunosuppression by naturally regulating the immune response and promoting tolerance to the graft. In a number of early clinical trials, polyclonal Treg therapy has demonstrated efficacy in maintaining graft function. However, optimal Treg immunotherapy should employ alloantigen-reactive rather than polyclonally-reactive Tregs to ensure both safety and enhanced specificity to the transplant.

Aim: Several approaches have been reported for the selective expansion of alloantigen-reactive Tregs, but none have demonstrated effective generation at a practical scale for clinical use. This study aimed to develop an effective method to rapidly expand functional human alloantigen-reactive Tregs.

Methods: $CD4^{+}CD25^{hi}CD127^{lo}$ human Tregs were flow sorted and stimulated *ex vivo* with allogeneic immature dendritic cells (iDCs). Cells were subsequently expanded by alloantigen stimulation for two weeks, followed by one week of polyclonal stimulation.

Results: Using *in vitro* suppression assays, alloantigen-reactive Tregs were found to be superior suppressors of effector cells and revealed potent allo-specific inhibition in comparison with polyclonally-expanded Tregs. Alloantigen-reactive Tregs maintained a high expression of Treg-specific and functional markers after expansion. Cytokine analysis revealed that alloantigen and polyclonal expanded Tregs express distinct pro-inflammatory cytokine profiles. Assessment of the T cell receptor repertoire revealed a restricted clonal expansion in alloantigen-reactive Tregs compared with polyclonally-expanded Tregs.

Conclusions: Our results suggest that the generation of alloantigen-reactive Tregs with definable allo-specificity is technically feasible. This methodology may provide a practical and GMP-compatible technique for alloantigen-reactive Treg generation without genetic manipulation.



Presenting author details
Full name: Alaa Ibrahim Alzhrani
Contact number: +447933936801
Twitter account: @Alzahrani_alaa
Email: Alaa_alzahrani@hotmail.co.uk
Session: Transplant Immunology
Category: (Oral presentation)

Biography
Last year DPhil candidate at Nuffield Department of Surgical Science, Oxford University. MSc degree in Immunology and Allergy, Nottingham University. BSc in Biomedical Sciences, King Saud University.