A124 HUMAN INDUCED CD4⁺CD25⁺FOXP3⁺ REGULATORY T CELLS ARE SUPPRESSIVE IN VITRO, BUT FAIL TO SUPPRESS INFLAMMATION IN VIVO

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Background Regulatory T cells (Treg) are important to maintain immune homeostasis. Presence of Treg correlates with a favourable disease course in juvenile idiopathic arthritis (JIA) patients. A distinction within the Treg population can be made between naturally occurring Treg (nTreg), which are derived from the thymus, and peripherally induced Treg (iTreg). Induction of Treg in the periphery is a promising way to modulate diseases like JIA, since it is easier to obtain high numbers of cells. However, the functionality of activation-induced CD4⁺CD25⁺FOXP3⁺ Treg has been highly debated in the last few years. Furthermore, in vivo induction of Treg by TCR or co-stimulation could induce a cytokine storm. Therefore, it is important to carefully monitor iTreg functionality.

Objectives In this study, the authors tested whether human iTreg suppress immune responses in vitro, and in vivo in a humanised mouse model of xenogeneic graft versus host disease (x-GvHD).

Methods CD4⁺CD25⁻ T cells were isolated from human PBMC and cultured with αCD3/αCD28 with or without interleukin-2 and TGFβ to obtain iTreg. CD4⁺CD25^{high} T cells were cultured with αCD3/αCD28, interleukin-2 and TGFβ to obtain nTreg. Supernatant was taken for Luminex analysis and cells were stained for Treg markers. Suppression assays were performed to determine suppressive capacity. RAG^{-/-} γc^{-/-} mice were sublethally irradiated and injected with clodronate liposomes to deplete phagocytosing cells. Next, human PBMC were injected with or without iTreg or nTreg from the same human donor. Mice were scored for x-GvHD during 9 weeks.

Results and conclusions The authors show here that induced Treg FOXP3 expression levels and suppressive capacity in vitro were comparable to nTreg. As expected, nTreg efficiently prevented acute x-GvHD. However, in contrast with nTreg, iTreg did not suppress disease. The results show that polyclonally induced Treg display no suppressive capacity in x-GvHD, due to a quick loss of FOXP3 in vivo. This underscores the importance to use humanised mouse models for validation of data regarding iTreg function obtained in in vitro assays, before proceeding to application in patients.