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Title : TNF-α priming of human regulatory T cells does not increase their ability to prevent xenogeneic graft-versus-host disease

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Introduction: Graft-versus-host disease (GVHD) is a frequent complication of allogeneic hematopoietic cell transplantation and is associated with significant morbidity and mortality. Numerous observations in experimental GVHD mouse models have demonstrated a role for Regulatory T Lymphocytes (Tregs) in the prevention and treatment of this disease when co-transplanted at high Treg/conventional T cell ratios (Edinger et al, Nature Medicine 2003; Leclerc et al, Blood 2016). In addition, prior studies have shown that TNF-α priming of Tregs enhanced their function and their ability to prevent GVHD in mouse models (Pierini et al, Blood 2016). In the current study, we aimed at assessing the impact of TNF-α priming of human Tregs in vitro and in a humanized mouse model of GVHD.

Material and methods: Firstly, we isolated Tregs from human peripheral blood mononuclear cells (PBMC) through immuno-magnetic selection, with a purity of 70-80%. Tregs were incubated with TNF-α at different concentrations for 48 hours. Changes in extracellular and intracellular markers were analyzed by spectral flow cytometry and gene expression was studied with single cell RNA sequencing. Secondly, we used a humanized mouse model in which Tregs ($0.5x10^6$ cells/mouse), previously primed or not with TNF in vitro for 48 hours, were injected to 2Gy irradiated NSG-HLA-A2 mice which had received CD-25-depleted human PBMC ($2x10^6$ cells/mouse) 48 hours before Tregs injection. We performed blood testing to follow human chimerism and clinical follow-up to detect signs of GVHD.

Results: Flow-cytometry experiments demonstrated that TNF-priming in vitro resulted in an overexpression of several activation markers such as HLA-DR, CTLA4, GARP, LAP, ICAM, CD69, ICOS and PD1. In contrast, TNFR2 was down-regulated as a result of internalization. In addition, single cell RNA sequencing demonstrated a significant up-regulation of genes involved in the non-canonical NF-κB pathway after exposure to TNF-α. This pathway can be activated by TNF-α through TNFR2, a receptor highly expressed by Tregs. A Western-blot on unprimed and primed Tregs confirmed that observation at the protein level. In vivo, the injection of a sub-optimal dose of Treg 48h after PBMC infusion tended to improve mice survival (HR=1.9, 95%CI 0.7-5.3, P=0.13, 9 mice per condition for a total of 27 mice). However, survival in mice given primed versus unprimed Treg were superimposable.

Discussion: In contrast with what has been observed in mouse-to-mouse models of GVHD, TNF-α priming of human Tregs failed to increase their ability to prevent GVHD in vivo when a suboptimal dose of Tregs was injected.

Conclusion: TNF-α successfully primed Tregs in vitro but failed to increase their ability to prevent GVHD in a humanized mouse model.

Keywords: graft-versus-host disease – T regulatory lymphocytes – TNF

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