5-Azacytidine and Rapamycin: different and synergistic effect on ex vivo expansion of natural human T Regulatory cells

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BACKGROUND: Natural T regulatory cells (Treg) are challenging to expand ex vivo, and this has severely hindered in vivo evaluation of their therapeutic potential. 5-Azacytidine (5-azaC) and Rapamycin (RAPA) are immunosuppressive drugs that promote selectively the expansion of CD4+CD25^{high}Foxp3+ regulatory T cells

OBJECTIVE: We investigated whether 5-azaC and RAPA could be used together to promote the ex vivo expansion of Tregs purified from adult human peripheral blood.

METHODS: CD4+CD25+ and CD4+CD25- T cells were isolated from PBMC of normal controls using Miltenyi beads and FACS ARIA sorting. These cells subsets were cultured in the presence of anti-CD3/CD28 antibodies and 200 IU/ml of IL2 for 2 weeks. RAPA (100nM) and 5-azaC (1µM) were added to half of the cultures. We monitored cell expansion and after harvest, the cell phenotype, gene expression, T suppression activity and Annexin V binding were determined and compared to values obtained with control cell culture and with freshly-separated CD4+CD25+ and CD4+CD25- T cells. Also we tested the absolute number of Treg in the several culture conditions during the time of expansion.
RESULTS: We found that 5-azaC helped maintain FOXP3 expression during the expansion process probably by promoting the conversion of T conventional (Tconv) in Treg, instead Rapa induces selectively apoptosis in Tconv cells and expansion in Treg. The addition of 5-azaC to RAPA treated cultures improved gene expression of FOXP3, CD25, STAT5 and TGF-B resulted in enhanced Treg expansion and suppressive activity. Also Rapa and 5-AzaC combination sustain Bcl-2 protein expression in Treg conferring resistance to apoptosis process.
CONCLUSION*:* 5-azaC may have utility in ex vivo expansion of human Tregs, not as a single agent, but in combination with RAPA. These data may considerably accelerate the development of immunotherapeutic approaches for the treatment of autoimmune disease or posttransplant alloreactions by the adoptive transfer of nTreg cells.