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Characterisation of Regulatory T cells in Normal Healthy Donors and Patients with Autoimmune Systemic Inflammatory Diseases

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Naturally arising CD4+CD25+ regulatory T cells (nTregs) are found to maintain peripheral tolerance, and depletion of these cells in mice leads to development of autoimmunity. In humans several studies have indicated a functional defect of Tregs or diminished cell numbers in patients with different autoimmune diseases which might contribute to their pathogenesis. However data regarding the phenotype of natural regulatory T cell in SLE patients are limited. Therefore we were interested to characterise natural regulatory T cells in patients with systemic lupus erythematosus (SLE) to further understand their role in its pathogenesis. Our next aim was to generate human Tregs from CD4+CD25neg T cells with properties comparable to nTregs in vitro and characterize those induced Treg to further explore their mechanism of immunosuppression.

SLE is a systemic autoimmune disease with an inflammatory phenotype, accompanied by high numbers of activated T cells (CD4+CD25++) in peripheral blood, which can lead to contamination of the nTreg population (CD4+CD25^{high}) and discrepancies regarding their numbers and function. To overcome this problem, we have chosen low expression of CD127 along with CD4+CD25^{high} as a marker of true nTregs for cell sorting. Thereby, we tudied the phenotype and functional role of CD4+CD25^{high}CD127$^{-/low}$ regulatory T cells in patients with SLE and Controls (Normal Donors (ND)). The SLE patients were divided into two groups according to their SLE disease activity index (SELENA-SLEDAI), a validated index of SLE activity, with one group comprising subjects with inactive SLE (SELENA-SLEDAI 3) and a second group comprising patients with active SLE (SELENA-SLEDAI $\geq$ 4). We observed no biologically significant difference in absolute numbers of CD4+CD25^{high}foxp3+ or CD4+CD25^{high}CD127$^{-/low}$ T cells and expression of foxp3 protein between active or inactive SLE patients or normal subjects. Proliferation of nTregs from SLE patients sorted for CD4+CD25^{high} vs. CD4+CD25^{high}CD127$^{-/low}$ significantly decreased from 9104 $\pm$ 1720 cpm to 2223 $\pm$ 351 cpm cpm, (ND: 2028$\pm$548 cpm to 802 $\pm$ 177 cpm), confirming reduced effector cell contamination. Notably, the suppressive activity of nTregs was intact in all groups. However, CD4+CD25^{-} responder T cells isolated from active SLE patients were significantly less sensitive towards the suppressive function of autologous or normal donor CD4+CD25^{high}CD127$^{-/low}$ nTreg cells. We observed that time points of 2 and 6 days were not optimal for measuring the amount of suppression due to an insufficient Tresp proliferation which was much lower as compared to the 4 day suppressor assays. Regulation of IFN-gamma levels in suppressor assays resembles the results of suppression of proliferation and use of anti-IFN-gamma Ab mainly caused a decrease in Tresp proliferation in both groups and did not impact on suppression. Preactivation of Tresp with IL-2 could not rescue the defective sensitivity in active SLE, but rather abrogated the Treg function in ND and aSLE. Furthermore, we found a significant inverse correlation between T responder cell suppressor sensitivity and lupus disease activity. We observed for the first time in human SLE, that an impaired sensitivity of Tresp towards the suppressive effects of an otherwise fully capable highly purified nTreg population leads to a defective suppression in active SLE patients

Our current results reveal that we were able to establish a culture system for generation and

expansion of human Treg from CD4+CD25-T cells and hereby autologous serum was observed to be optimal for maintaining FOXP3 expression and regulatory function. These "induced" Tregs (iTregs) showed potent suppressive activity, comparable to nTreg and their suppressive activity was already observed after three days maturation in culture. However, the functional capacity of iTregs was slightly lower than nTregs at higher dilutions in suppressor assays. The mode of suppression was contact dependent and TGF-beta or IL-10 were not involved in suppressor function. Phenotypical features of generated Tregs were almost similar to expanded nTregs in terms of expression of surface markers, such as GITR, CD62L HLA-DR, CD95 and the intracellular CTLA-4. A slight decrease in percentage of cells expressing FOXP3 Protein and mRNA in iTregs compared to nTregs was observed. Furthermore, FOXP3 expression by iTreg remained constant after a resting period of 48h in different concentrations of IL-2. We observed a slightly higher expression of T-bet in iTreg when compared to nTreg at day 5; it was further increasing until day 15. GATA-3 expression is quite in contrast to the T-bet i.e. nTreg were showing a relatively higher expression when compared to iTreg. In contrast to nTreg, however, a proportion of these iTreg was producing IFN-γ and IFN- γ production was not suppressed in responder T cells, while proliferation was. We observed a slightly inhibitory effect of immunosuppressants on expansion of iTregs but iTreg matured in presence of immunosuppressants were still able to suppress the responder T cell proliferation *in vitro.* We also observed that the number of target T cells which are undergoing apoptosis are not increased in presence of induced Treg or natural Treg. Finally, in contrast to natural Treg, regulation of Tresp proliferation by generated T cells is observed only in medium containing serum despite their generation either in serum or serum free medium and presence of FOXP3 among them.