Depletion of T regulatory cells through selection of CD127-positive cells results in a population enriched in memory T cells: implications for anti-tumor cell therapy

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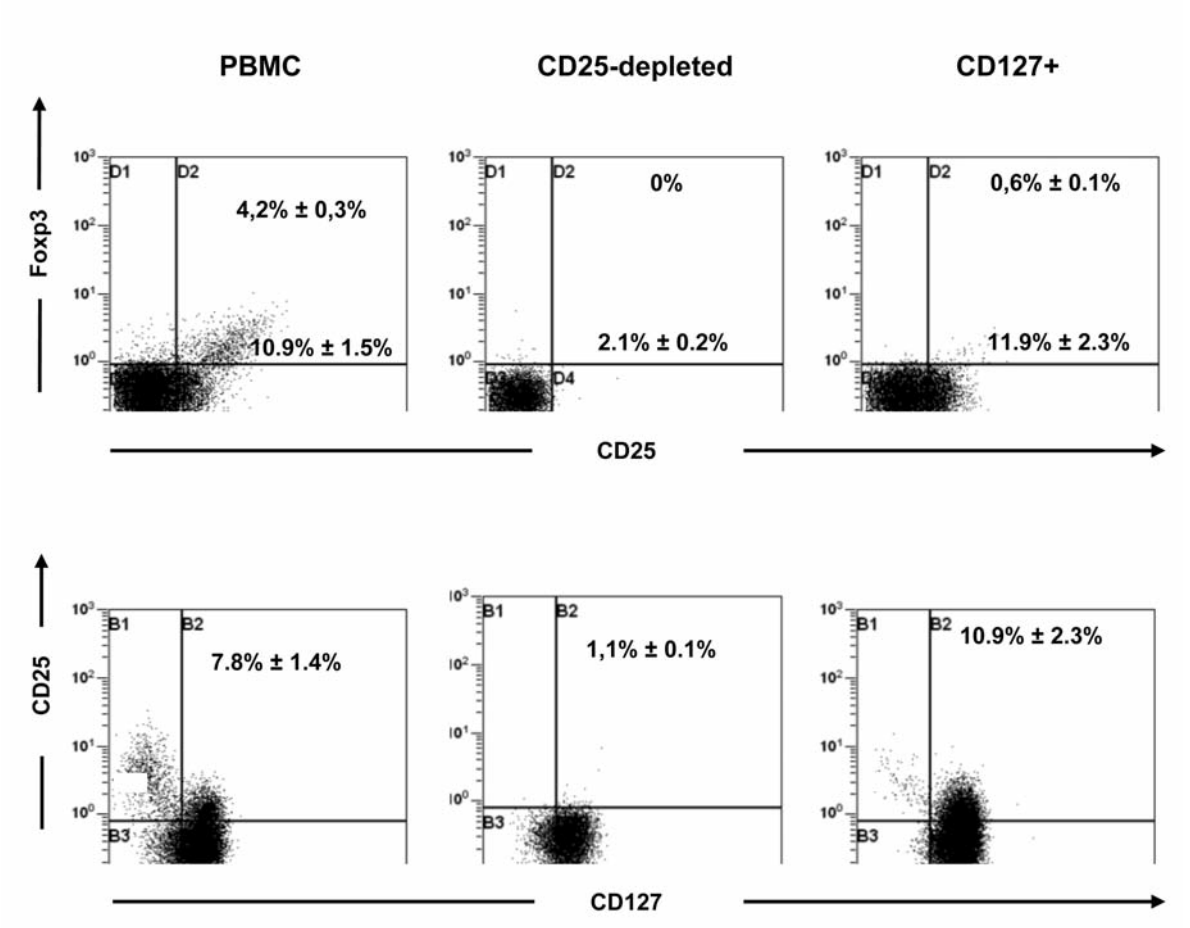
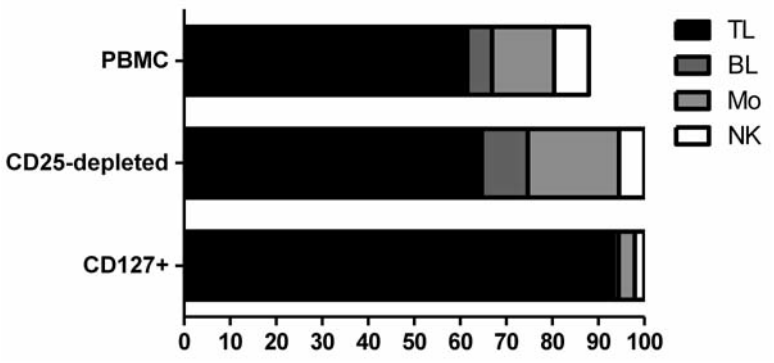
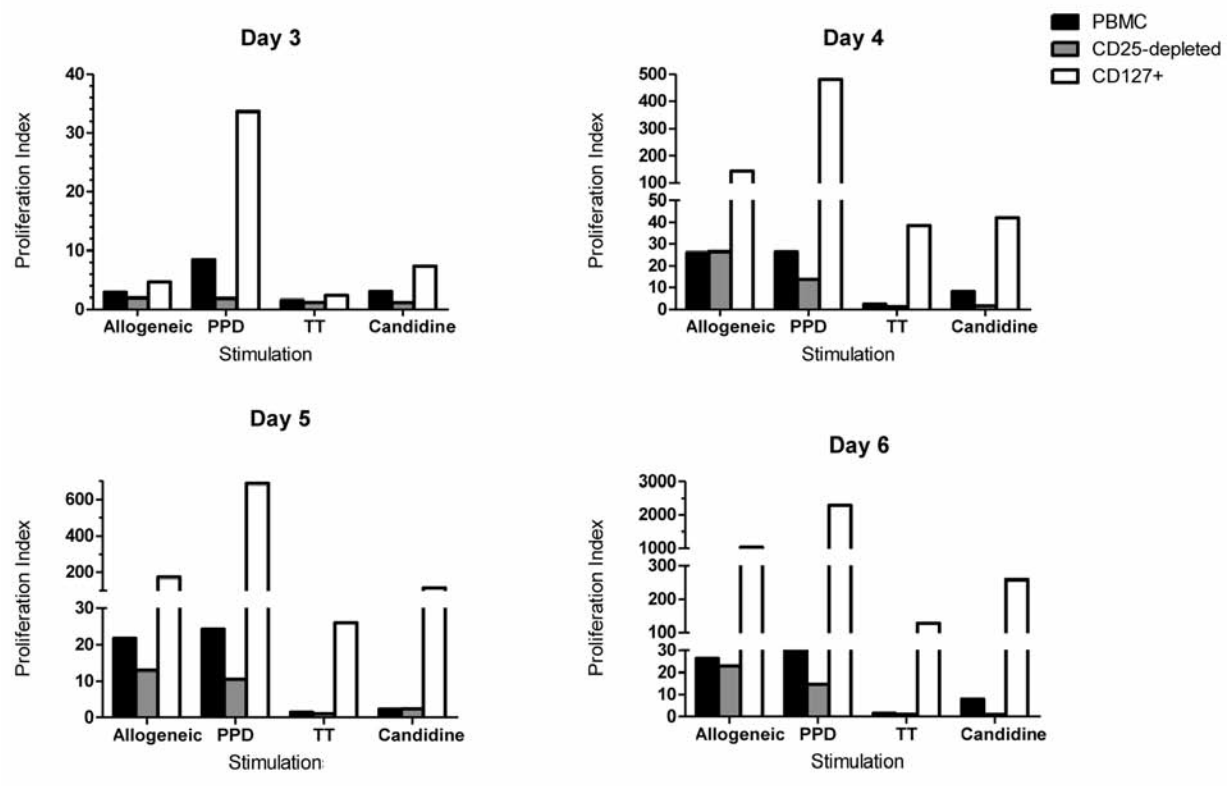


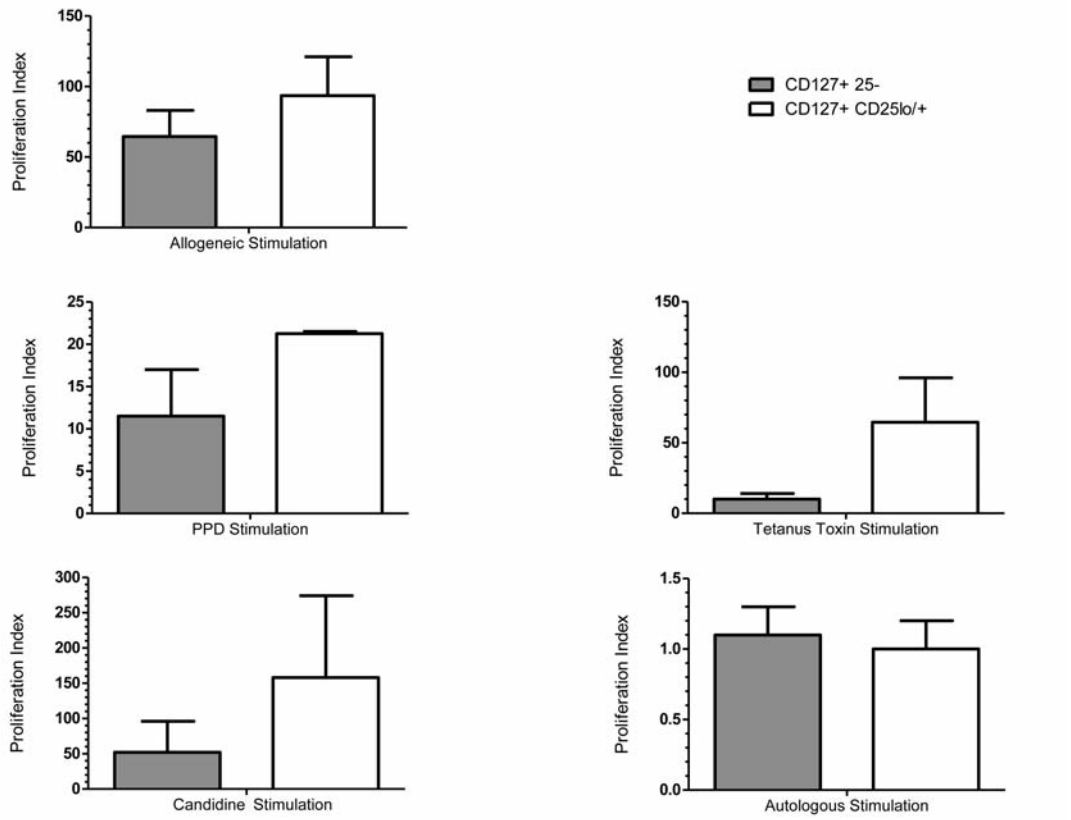
Online Supplementary Figure S1. Phenotypic characterization of cells following Treg depletion. Freshly isolated PBMC, CD25-depleted and CD127-selected cells were gated into $CD3^+CD4^+$ cells. Top panels identify Treg through the $CD25^{high}Foxp3^+$ phenotype and conventional activated T cells through $CD25^+$ $Foxp3^-$. Bottom panels identify conventional activated T cells through the $CD25^+$ $CD127^+$ phenotype. Data shown are representative of the 14 independent experiments in Figure 2.



Online Supplementary Figure S2. Cell content modifications following Treg depletion. The cell content was evaluated in non-manipulated and in Treg depleted PBMC. Stacked bars show the percentage of T lymphocytes (TL), B lymphocytes (BL), monocytes (Mo), and natural killer cells (NK) in non-manipulated PBMC and Treg depleted cells. The figure represents the results of one out of three independent experiments.



Online Supplementary Figure S3. Kinetics of the immune response of cells following Treg depletion. PBMC and Treg-depleted cell populations were tested for their capacity to respond to allogeneic or pathogen-specific stimulation on day 3, 4, 5 or 6. Histograms represent proliferation of PBMC (black), CD25-depleted (gray) and CD127-positively selected cells (white) expressed as counts per minute (cpm). The figure shows the results of one experiment carried out in triplicate. Data shown are mean ± SEM. PPD: purified protein derivative; TT: tetanus toxoid.



Online Supplementary Figure S4. Comparative *in vitro* functional responses of FACS-sorted CD25^{low}CD127^{+} and CD25^{-}CD127^{+} cells. CD127^{+} CD25^{-} and CD127^{+} CD25$^{lo/+}$ cells were first obtained by separation of unmanipulated T cells from PBMC using the Pan T Cell Isolation Kit II, an LS Column, and a MidiMACS separator according to the manufacturer's protocol (Miltenyi Biotec, Germany). CD3^{+} cells were then stained with CD127-FITC, CD25-PE and CD127^{+}CD25^{-} and CD127^{+}CD25$^{lo/+}$ cells were sorted by flow cytometry using a FACSAria (BD Biosciences) to a purity of >97%. Sorted cell-populations were tested for their capacity to respond to allogeneic, pathogen-specific or autologous stimulations after 6 days of culture. Histograms represent the proliferation index calculated as proliferation (cpm) of stimulated cells divided by proliferation of non-stimulated cells. Data shown are mean ± SEM of two independent experiments from two healthy donors. PPD: purified protein derivative; TT: tetanus toxoid.