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Large scale *in vitro* expansion of polyclonal human CD4⁺CD25^{high} regulatory T cells

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CD4⁺CD25⁺ regulatory T (T_{reg}) cells are pivotal for the maintenance of self-tolerance and their adoptive transfer protects from autoimmune diseases and pathogenic alloresponses after solid organ or bone marrow transplantation in murine model systems. *In vitro*, human CD4⁺CD25⁺ T_{reg} cells display similar phenotypic and functional characteristics as murine CD4⁺CD25⁺ T_{reg} cells, namely hyporesponsiveness to TCR stimulation and suppression of CD25⁺ T cells. Thus far, the detailed characterization and potential clinical application of human CD4⁺CD25⁺ T_{reg} cells was hampered by their paucity in peripheral blood and the lack of appropriate expansion protocols. Here we describe the up to 40,000-fold expansion of highly purified human CD4⁺CD25^{high} T cells *in vitro* through the use of artificial APC for repeated stimulation via CD3 and CD28 in the presence of high dose IL-2. Expanded CD4⁺CD25^{high} T cells were polyclonal, maintained their phenotype, exceeded the suppressive activity of freshly isolated CD4⁺CD25^{high} T cells and showed characteristics of central memory T cells. The ability to rapidly expand human CD4⁺CD25^{high} T_{reg} cells large scale will not only facilitate their further exploration but also accelerate their potential clinical application in T cell-mediated diseases and transplantation medicine.