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The study on the expansion and function of Tregs from human PBMCs

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Objectives : Regulatory T cells (Tregs) constitute a vital subgroup within CD4+ T cells. These cells play a pivotal role in inducing immune tolerance, preserving immune homeostasis, and mitigating autoimmune diseases. Typically, Tregs are present in small numbers under normal physiological conditions. The current study aims to devise an effective method for expanding human peripheral blood Tregs in vitro and subsequently analyzing the phenotype, purity, and function of these expanded Treg cells.
Methods : Peripheral blood samples were collected from 11 healthy donors. Tregs were isolated from peripheral blood mononuclear cells (PBMCs) using magnetic-activated cell sorting (MACS) based on the expression of CD4+ and CD25+ markers. An optimized culture system was employed for the amplification of Tregs. The in vitro amplification efficiency of Treg cells was assessed to evaluate the expression levels and purity of Treg cell-specific surface markers across various culture cycles. The analysis of Tregs involved using Flow cytometry to analyze various target markers, and Treg-related genes were analyzed using RT-PCR methods.
Results : Treg cells were isolated via magnetic sorting and cultured for 11 days. The Tregs/PBMC ratio significantly differed between younger (<40 years) and older (>60 years) groups. Expanded Tregs showed clustering and increased geometrically from day 7. Flow cytometry revealed CD4+/CD25+ and CD25+/Foxp3+ cells increased 157- and 60-fold, respectively. Gene expression of CD25, Foxp3, IL-10, and CTLA-4 was upregulated. Notably, Tregs treated with ASC secretome showed increased CD25+, Foxp3+, and CTLA4+ expression, warranting further study on its proliferative effects.
Conclusions : We have successfully developed a technical protocol for generating a substantial quantity of Tregs with high efficiency in vitro. These expanded Tregs consistently maintain FOXP3 expression and demonstrate potent immune suppression. This finding holds significant promise for adoptive Treg therapy in the treatment of graft-versus-host disease and autoimmune disorders.