**Effective and reliable in vitro generation of human \( \text{T}_{\text{H}17} \) cells**

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**Introduction**

\( \text{T}_{\text{H}17} \) cells are a unique lineage of CD4⁺ T helper cells, which characteristically secrete IL-17 at high levels and, to a lesser extent, IL-22. \( \text{T}_{\text{H}17} \) cells are involved in immune responses against extracellular pathogens, such as *Candida*. \( \text{T}_{\text{H}17} \) cells also play a critical role in the pathogenesis of autoimmune diseases and other atopic and chronic inflammatory disorders.

\( \text{T}_{\text{H}17} \) cell differentiation is orchestrated by multiple cytokines. Naïve human T helper cells can be induced to differentiate *in vitro* into \( \text{T}_{\text{H}17} \) cells by exposure to TGF-β and proinflammatory cytokines, particularly IL-1β and IL-6, whereas IL-23 promotes survival and proliferation of \( \text{T}_{\text{H}17} \) cells. The possibility of generating a highly enriched, consistent population of \( \text{T}_{\text{H}17} \) cells *in vitro* provides opportunities to investigate the biology of \( \text{T}_{\text{H}17} \) cells in detail.

**Materials and methods**

**Recombinant cytokines and neutralizing antibodies**

\( \text{T}_{\text{H}17} \)-polarizing cytokines, i.e., human IL-1β, IL-6, IL-23, and TGF-β1, from Miltenyi Biotec (MACS® Cytokines) were tested against the same cytokines from other providers. Functional-grade antibodies against IFN-γ (clone 45-15) and IL-4 (clone 7A3-3) were from Miltenyi Biotec.

**Isolation of naive human T cells**

Human PBMCs were prepared from peripheral blood of healthy donors by density gradient centrifugation. Naïve CD4⁺CD45RA⁺ T cells were purified from PBMCs using the Naïve T Cell Isolation Kit, human (Miltenyi Biotec).

**\( \text{T}_{\text{H}17} \) cell generation**

Naïve CD4⁺ T cells were seeded in 24-well plates (1×10⁶ per well) and cultured in a serum-free medium. Cells were stimulated by using the T Cell Activation/Expansion Kit, human (Miltenyi Biotec), which is based on MACS®Bead™ Particles loaded with CD2, CD3, and CD28 antibodies. The bead-to-cell ratio amounted to 1:2.

Cells were cultured in the presence of the \( \text{T}_{\text{H}17} \)-polarizing cytokines, IL-1β (20 ng/mL), IL-6 (30 ng/mL), IL-23 (30 ng/mL), and TGF-β1 (2.25 ng/mL), in addition to Anti-IFN-γ (1 µg/mL) and Anti-IL-4 (2.5 µg/mL) antibodies. Cells were cultured for 7 days at 37 °C, in an atmosphere of 5% CO₂, without any media exchange. Enzyme-linked immunosorbent assays (ELISA) were performed as described.

**ELISA**

Cell culture supernatants were collected, filtered, and measured for their content of IFN-γ, IL-4, IL-17, IL-22, by using ELISA (R&D Systems) according to the manufacturer’s protocol.
Results and conclusion

Here we show that a combination of recombinant human TGF-β1, IL-23, and the proinflammatory cytokines IL-1β and IL-6 effectively drives differentiation of isolated naive human CD4+ T cells into Th17 cells. In addition to IL-17 production, the hallmark of Th17 cells, these Th17-polarizing conditions also induced low-level production of IL-22 (Fig. 1). By contrast, IFN-γ and IL-4, which are characteristic of Th1 and Th2 cells, respectively, could not be detected.

The quality of cell culture ingredients dramatically influences cell behavior. Consistent, high-quality products are essential for reliable and functionally relevant cell culture results. We compared recombinant cytokines from different providers. Premium-grade cytokines from Miltenyi Biotec showed at least equal performance compared to other commercially available cytokines and consistently generated Th17 cells in vitro under the reported Th17-polarizing conditions.

Figure 1: Naive human CD4+ T cells were stimulated with MACS Bead Particles loaded with CD2, CD3, and CD28 antibodies, in the presence of a Th17-polarizing cytokine cocktail (IL-1β, IL-6, IL-23, and TGF-β1), in serum-free medium. Concentrations of IL-17, IL-22, IFN-γ, and IL-4 in cell culture supernatants were determined by ELISA on day 7 (n=2, independent donors). Error bars represent SEM.

References