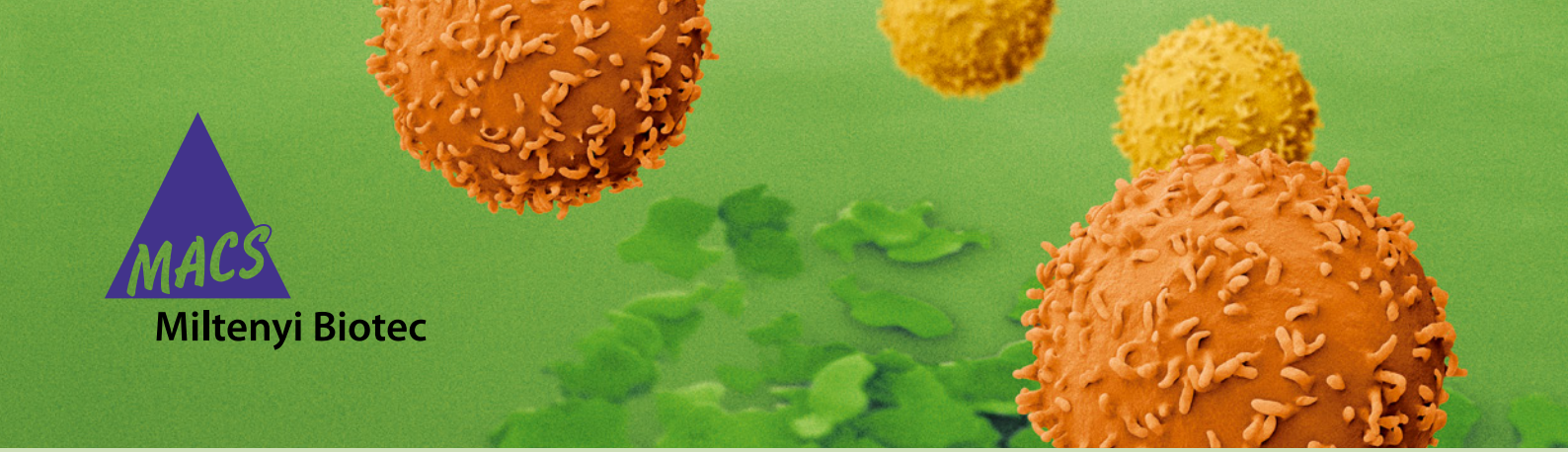

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Report

TH17 cell differentiation

Effective and reliable *in vitro* generation of human TH17 cells

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Introduction

TH17 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. TH17 cells are involved in immune responses against extracellular pathogens, such as *Candida* [1].
TH17 cells also play a critical role in the pathogenesis of autoimmune diseases and other atopic and chronic inflammatory disorders [2].
TH17 cell differentiation is orchestrated by multiple cytokines. Naive human T helper cells can be induced to differentiate *in vitro* into TH17 cells by exposure to TGF-β and proinflammatory cytokines, particularly IL-1β and IL-6, whereas IL-23 promotes survival and proliferation of TH17 cells [3,4].
The possibility of generating a highly enriched, consistent population of TH17 cells *in vitro* provides opportunities to investigate the biology of TH17 cells in detail.

Materials and methods

Recombinant cytokines and neutralizing antibodies

TH17-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. Naive $CD4^+CD45RA^+$ T cells were purified from PBMCs using the Naive T Cell Isolation Kit, human (Miltenyi Biotec).

TH17 cell generation

Naive $CD4^+$ T cells were seeded in 24-well plates (1×10^6 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 MACSiBead™ 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 TH17-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% CO_2, 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.

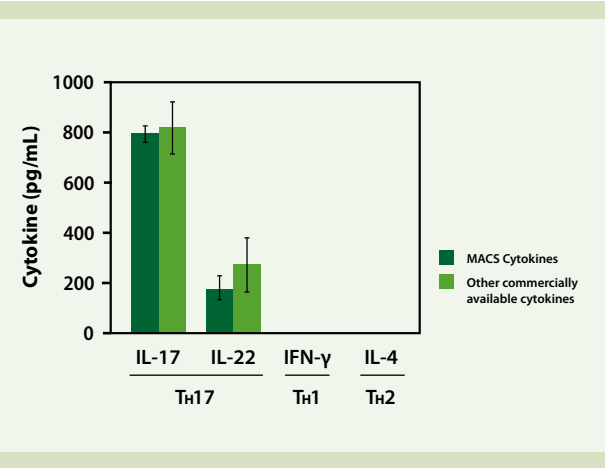

Figure 1: Naive human CD4$^+$ T cells were stimulated with MACSiBead Particles loaded with CD2, CD3, and CD28 antibodies, in the presence of a TH17-polarizing cytokine cocktail (IL-1β, IL-6, IL-23, and TGF- β1), in serum-free medium. Concentrations of IL-17, IL-22, IFN-γ, and IL-4 in cell culture supernatants were determined by ELISA on day 7 (n=2, independent donors). Error bars represent SEM.

References

1. Eyerich, K. *et al.* (2008) J. Invest. Dermatol. 128: 2640–2645.
2. Korn, T. *et al.* (2009) Annu. Rev. Immunol. 27: 485–517.
3. Manel, N. *et al.* (2008) Nat. Immunol. 9: 641–649.
4. Volpe, E. *et al.* (2008) Nat. Immunol. 9: 650–657.

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