In vitro differentiation of human Th-17 CD4+ T cells

Nicolas Manel (manel@saturn.med.nyu.edu)
New York University

Method Article

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Abstract

Introduction

Human CD4+ Th-17 cells produce inflammatory cytokines and have been implicated in the development of several inflammatory pathologies. The transcription factor RORgammaT is though to establish Th-17 cell differentiation. Expression of IL-17A is a hallmark of Th-17 cells. We have used RORgammaT overexpression as an approach to identify factors that induce Th-17 cell differentiation. This led us to the observation that TGF-beta is essential for induction of RORgammaT. However, unknown factors present in serum inhibit Th-17 cell differentiation. Thus, it is important to cultivate cells in serum-free conditions in order to generate Th-17 cells from naive CD4+ T cells. In serum-free conditions, we found that a combination of TGF-beta, IL-1beta and either IL-6, IL-21 or IL-23 is able to induce Th-17 cell differentiation. Here is described a procedure to generate human Th-17 cells from naive CD4+ T cells isolated from adult or cord blood.

Reagents

- Miltenyi human CD4 microbeads - Fluorescently labelled ant-CD3, anti-CD4, anti-CD45RA, anti-CD25, anti-HLA-DR, anti-IL-17 - Recombinant human IL-1beta, IL-23, IL-2, TGF-beta - Serum-free media \(e.e Lonza XVIVO-20) - Anti-CD3/CD28 activation beads - Phorbol 12-myristate 13-acetate \(PMA) - Ionomycin - BD GolgiStop - BD Perm/Fix and BD Perm/Wash Intracellular staining buffers

Equipment

- Magnetic cell separator - Cell sorter - Flow cytometer

Procedure

DAY 1 1. Isolate mononculear cells from adult or cord blood on a FicollPAQUE gradient 2. Use Miltenyi human CD4+ beads and procedure to isolate CD4+ T cells 3. Isolate naive CD4+ T cells by cell storing. Human naive CD4+ T cells are typically defined as CD3+CD4+CD45RA+CD25-HLA-DR- but more complex sorting schemes can been utilized \(1). **TIP**: Coat tubes with serum-free media for harvesting cells during sort. 4. Count cells and resuspend in fresh serum-free media at a concentration of 250,000 to 500,000 cells per mL 5. Add 10 U/ml of IL-2, 10 ng/mL of IL-1beta, 10 ng/ml of IL-23, 1 µg/ml of anti-IL-4, 1 µg/mL of anti-IFNgamma and anti-CD3/CD28 activation beads at a ratio of 1 bead per cell 6. In U-bottom 96-well plates, aliquote 200 µL per well 7. Add an increasing concentration of TGF-beta to a serie of four wells : 0, 0.1, 1 and 10 ng/mL **TIP**: TGF-beta is a highly hydrophobic protein and its activity can be variable. Thus, it is important to titrate TGF-beta systematically in each experiment. **DAY 3** **TIP**: At day 3, cells spontaneously gather to the center of each well and should be visible macroscopically. 8. Spin plates, remove media and replace with fresh media containing all cytokines and antibodies **DAY 5** 9. Split each well in half 10. Spin plates, remove media and replace with fresh

media containing all cytokines and antibodies **DAY 6** 11. Activate cells for 5 hours in the presence of 50 ng/ml of PMA, 500 ng/ml of ionomycin and 1x of BD GolgiStop 12. Fix cells in BD Perm/Fix and proceed for intracellular staining of IL-17A and IFNgamma in BD Perm/Wash buffer 13. Analyse cells by flow cytometry **TIP**: In the presence of TGF-beta, 1% to 15% of IL-17A+ cells are typically observed

References

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