Generation of Human IL-17–Producing Cells (Th17-like Cells) in Vitro

Introduction

T lymphocytes play key roles in protective adaptive immune responses to a variety of pathogens. Regulation of T-cell responses is the key to maintaining immune balance. This balance can be disrupted, either when T cells recognize or respond to self-antigens, as in the case of autoimmune diseases, or when they fail to respond effectively to microbial pathogens and tumors. BD Biosciences offers an extensive portfolio of reagents and instruments for high resolution multiparameter analysis of different CD4 T-cell subsets that include Th1, Th2, Th9, and Th17, along with T follicular helper (Tfh) cells and regulatory T cells. We also offer tools to assess accessory cells, for example, antigen-presenting dendritic cells that affect the function of CD4 T-cell subsets.

Interleukin 17 (also known as IL-17 or IL-17A) is the original member of the IL-17 family, which also includes IL-17B, IL-17C, IL-17E (also called IL-25), and IL17-F. IL-17F displays the highest degree of homology with IL-17A. IL-17 is the signature cytokine of a unique subset of $CD4^+$ T cells called Th17 cells.[1] Additional cellular sources of IL-17A include $CD8^+$ αβ T cells, γδ T cells, LTi cells, neutrophils, and eosinophils.[1,2] IL-17 also is produced by NKT cells upon T-cell antigen receptor (TCR) stimulation.[2]

IL-17 binds to and signals through IL-17 receptor (IL-17R) complexes composed of type 1 transmembrane IL-17RA and IL-17RC protein subunits that are widely expressed on epithelial cells, endothelial cells, and fibroblasts. The ligation of IL-17/IL-17R results in the release of inflammatory mediators, including IL-1, IL-6, IL-8, IL-23, TNF, and several chemokines to further stimulate the inflammatory cascade. The following table summarizes the key characteristics of differentiation and functions of Th17 cells.

Table 1. Key characteristics of Th17 cells

Main function	Recruit and enhance functions of neutrophils at sites of inflammation
Pathogens targeted	Fungi and extracellular bacteria
Undesirable effects	Organ-specific autoimmune disease
Extracellular markers	CD4, CCR6, CD126, CD161
Differentiation cytokines	TGF-β1, IL-6, IL-1, IL-21, IL-23
Effector cytokines	IL-17A, IL-17F, IL-21, IL-22, IL-26, TNF, CCL20
Transcription factors	RORγt, Stat3

Procedural Notes

- Method 1 is useful as a rapid test for potential IL-17 production by activated lymphoid cells. This protocol is not intended as a culture method for generating differentiated Th17-like cells or cloning of Th17 cells. For the latter cell types, we recommend using Method 2, the long-term Th17 cell polarization culture protocol.

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Method 1: Generating IL-17–Producing Peripheral Blood Mononuclear Cells (PBMCs) using PMA and Ionomycin Stimulation

Reagents and Antibodies

Full Reagent Name	Short Name	Catalog Number
RPMI-1640 containing 10% FCS	Culture medium	
Sterile 1X Dulbecco's PBS	1X PBS	Gibco 14040
Ficoll-Paque™ Plus	Density gradient	GE 17-1440-02
BD Pharm Lyse™ lysing buffer	Lysing solution	555899
Phorbol 12-Myristate 13-Acetate	PMA	Sigma P-8139
Ionomycin	Ionomycin	Sigma I-0634
BD Pharmingen™ stain buffer (BSA)	Stain buffer	554657
BD GolgiStop™ protein transport Inhibitor (Monensin)	Transport inhibitor	554724
BD Cytofix™ fixation buffer	Fixation buffer	554655
BD Perm/Wash™ buffer	Perm/Wash buffer	554723

Procedure

1. Prepare a single-cell suspension of PBMCs by density gradient centrifugation.
2. If residual RBCs are present in the PBMCs, lyse them using lysing solution.
3. Activate the cells by placing them, with 50 ng/mL of PMA and 1 µg/mL of ionomycin, in culture medium and incubating for 5 hours at 37°C.
4. Resuspend the cells in fixation buffer at 4°C for 10 to 20 minutes.
5. Wash the cells twice in stain buffer.
6. Resuspend the cells either in stain buffer (for storage at 4°C for up to 72 hours) or in 90% FCS/10% DMSO (for storage at −80°C for longer periods of time).
7. When ready to continue: for cells stored at 4°C, pellet by centrifugation and remove the supernatant; for frozen cells, wash twice in stain buffer to remove the DMSO.
8. Resuspend the cells in 1X Perm/Wash buffer for 15 minutes at 4°C.
9. Pellet the cells by centrifugation.
10. Incubate the cells with fluorescent antibodies at 4°C for 20 to 30 minutes in the dark.
11. Wash the cells twice with 1X Perm/Wash buffer and resuspend them in stain buffer before flow cytometric analysis.
12. Once the staining is completed, acquire the cells, making sure to collect as least 15,000 $CD4^+$ cell gated events for data analysis.

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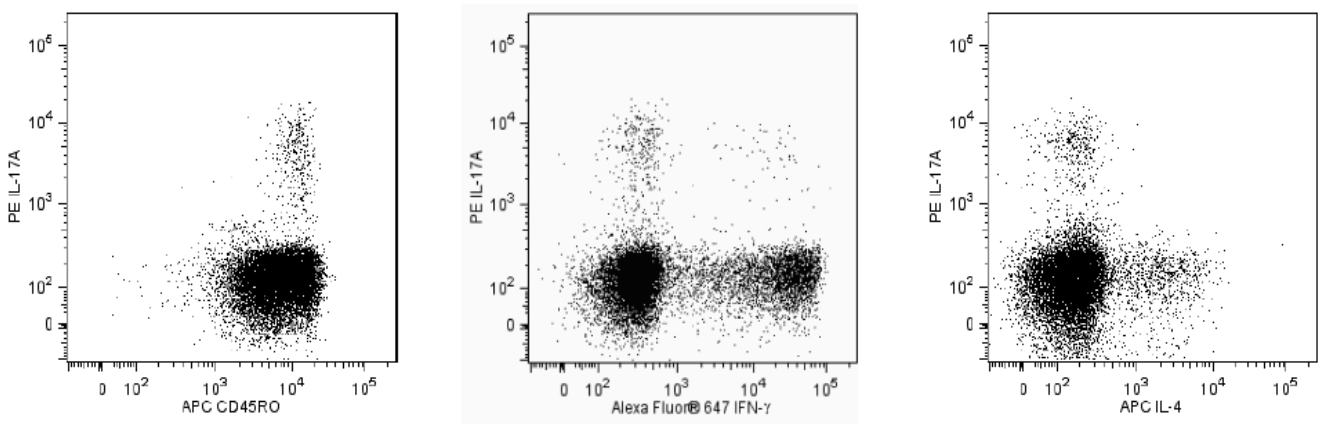

Figure 1. Multicolor flow cytometric analysis of human IL-17A expressed in stimulated CD4$^+$ peripheral blood T cells. Human PBMCs were stimulated with PMA/ionomycin in the presence of transport inhibitor for 5 hours. Cells were then fixed and permeabilized using fixation and Perm/Wash buffers, followed by staining with PE anti-human IL-17A, PerCP anti-human CD4, and either APC anti-human CD45RO or Alexa Fluor® 647 anti-human IFN-γ.

Method 2: Generating Polarized Human Th17-like Cells (Long-Term Cultures)

Reagents and Antibodies

Full Reagent Name	Short Name	Catalog Number
Ficoll-Paque™ Plus	Density gradient	GE 17-1440-02
RPMI-1640 containing 10% FCS	Culture medium	
Sterile 1X Dulbecco's PBS	1X PBS	Gibco 14040
BD Pharm Lyse™ lysing buffer	Lysing solution	555899
BD GolgiStop™ protein transport inhibitor (Monensin)	Transport inhibitor	554724
NA/LE Anti-Human CD3e antibody, clone UCHT1	Anti-CD3	555329
NA/LE Anti-Human CD28 antibody, clone CD28.2	Anti-CD28	555725
Anti-Human IL-4 antibody, clone MP4-25D2	Anti-IL-4	554481
Anti-Human IFN-γ antibody, clone B27	Anti-IFN-γ	554698
Recombinant IL-1β protein	IL-1β	554602
Recombinant IL-6 protein	IL-6	550071
Recombinant IL-23 protein	IL-23	R&D Systems 1290-IL-010
Recombinant TGF-β1 protein	TGF-β1	354039
BD Pharmingen™ stain buffer (BSA)	Stain buffer	554657
BD Cytofix/Cytoperm™ fixation/ permeabilization buffer	Fix/perm solution	554722
BD Perm/Wash™ buffer	Perm/Wash buffer	554723

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Procedural Notes

- Cells can be cultured for up to 21 days depending on the researcher's needs. However, there is a significant increase in the numbers of IL-17–producing cells starting at day 6.
- Refresh cells cultured for extended periods of time with new medium every other day.

Procedure

1. Prepare a single-cell suspension of human PBMCs by density gradient centrifugation.
2. If residual RBCs are present in the PBMCs, lyse them using lysing solution.
3. Resuspend the PBMCs in culture medium at a density of 0.5–1.0 x 10^6 cells/mL.
4. Add the cells to a tissue culture plate pre-coated with anti-CD3 antibody (10 µg/mL) together with soluble anti-CD28 antibody (1 µg/mL), IL-6 (10 ng/mL), IL-1β (10 ng/mL), TGF-β1 (5–10 ng/mL), IL-23 (10 ng/mL), and the neutralizing antibodies anti-IL-4 (10 µg/mL) and anti-IFN-γ (10 µg/mL) in culture medium for the desired number of days.
5. When culturing the cells for extended period of time, the cells can be refreshed with new medium every other day and on days 10–14 (or up to 21 days). On the day of the harvest, restimulate the cells with PMA (50 ng/mL) and ionomycin (1 µg/mL) in the presence of transport inhibitor (4 µL for every 6 mL of the culture medium) for 5 hours, and harvest the cells for analysis or freezing.
6. Proceed with the staining, acquisition, and analysis steps used in Method 1, starting at step 10.

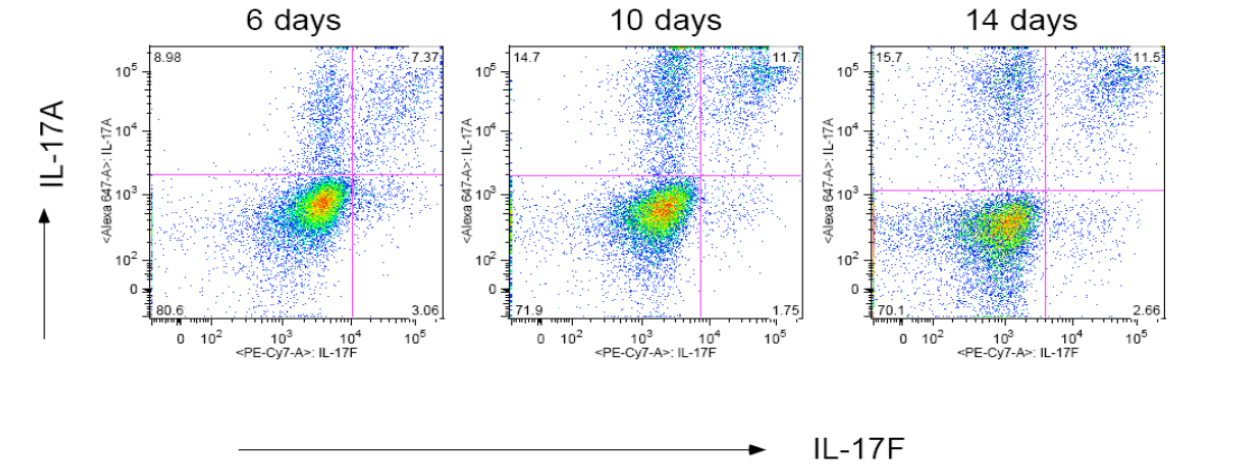

Figure 2. Multicolor flow cytometric analysis of human IL-17A expressed in stimulated CD4$^+$ peripheral blood T cells. Human PBMCs, along with anti-CD3 and anti-CD28 antibodies and recombinant proteins, were cultured for 14 days to generate Th17-like polarized CD4 T cells. The cells were harvested at appropriate times as indicated. The cells were further fixed and permeabilized using fix/perm solution followed by staining with Alexa Fluor® 647 anti-human IL-17A (Cat. No. 560437, 560439).

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References

1. Gaffen SL. An overview of IL-17 function and signaling. *Cytokine*. 2008;43:402-407.
2. Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. *Nature.* 2008;453:1051-1057.

Additional Reading

Veldhoen M, Hirota K, Christensen J, O'Garra A, Stockinger B. Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 cells. *J Exp Med*. 2009;206:43-49.

Laurence A, Tato CM, Davidson TS, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity*. 2007;26:371-381.

23-14721-02