

## Th9 Polarization of Mouse Splenocytes

### Introduction

Th9 cells are a subpopulation of T-helper cells, characterized to be involved in allergic diseases and resistance against intestinal nematodes. Unlike Treg cells, Th9 cells are not suppressive, as they can promote T cell proliferation along with other effector T cells. Th9 cells do not express any well-defined transcription factors like T-bet, GATA3, ROR $\gamma$ t and Foxp3, clearly differentiating them from Th1, Th2, Th17 and Foxp3+ iTreg populations. TGF- $\beta$  reprograms Th2 T-helper cells to lose their characteristic profile of IL-17 secretion and switch to IL-9 secretion. Differentiation of Th9 cells can be obtained by a combination of TGF- $\beta$  and IL-4. In addition, the neutralization of IFN- $\gamma$  is critical to drive the pathway to Th9 cells. Here we provide an effective protocol for the generation of mouse Th9 cells *in vitro*.

### Th9 Polarization of splenocytes

1. Coat 60  $\times$  15 mm of plastic petri dishes with anti-mouse CD3 $\epsilon$ , clone 145-2C11 (5  $\mu$ g/ml). Incubate at 37°C for 2 hours or 4°C overnight. Aseptically decant antibody solution from the plate. Wash plate 3 times with sterile PBS. Discard liquid.
2. Plate splenocytes at 1  $\times$  10<sup>6</sup>/ml. Culture cells for 3 days in presence of anti-mouse CD28, clone 37.51 (5  $\mu$ g/mL), recombinant TGF- $\beta$ 1 (10 ng/mL), recombinant mouse IL-4 (10 ng/mL), recombinant mouse IL-2 (20 ng/ml), and anti-mouse IFN- $\gamma$  (10  $\mu$ g/mL).
3. On day 3, wash cells once and then restimulate in complete medium with 500 ng/ml PMA and 500 ng/mL ionomycin, in the presence of Brefeldin A for 6 hours.
4. After harvesting, the cells are ready for staining.

## Materials

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile plastic petri dishes
- RBC Lysis Buffer (cat. # 420301)
- Anti-mouse CD3 $\epsilon$ , clone 145-2C11 (LEAF<sup>™</sup> format, cat. # 100314)
- Anti-mouse CD28, clone 37.51, (LEAF<sup>™</sup> format, cat. # 102112)
- Anti-mouse IFN- $\gamma$  (LEAF<sup>™</sup> format, cat. # 505812)
- Recombinant mouse IL-4 (cat. # 563202)
- Recombinant human TGF- $\beta$ 1 (carrier-free) (cat. # 580702)
- Recombinant mouse IL-2 (cat. # 575402)
- Brefeldin A (cat. # 420601)
- PMA (cat. # P8139 from Sigma)
- Ionomycin (cat. # I0634 from Sigma)

## References

1. Soler, D. *et al.* 2006. *J Immunol.* 117:6940.
2. Schmitt, E. *et al.* 1994. *J Immunol.* 153:3989.
3. Veldhoen, M. *et al.* 2008. *Nat Immunol.* 9:1341.
4. Darhalhon, V. *et al.* 2008. *Nat. Immunol.* 9:1347.