In vitro Differentiation of Mouse Th0, Th1 and Th2 from Naïve CD4 T Cells

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Summary of Q&A (last updated on 9/19/2016)

During the Experiment

Q#1. *Why transfer to a new plate in step 5? Does new plate need to be coated with anti-CD3 and add anti-CD28? Do I need to wash the cells?*

A. Because anti-CD3 antibody will cause cell death, it is necessary to transfer cells to new plates, that's also why anti-CD3 is plate-bound at beginning so that no anti-CD3 will be transferred together with cells and cells don't need to be washed. Check step 5 in the protocol: "transfer the culture to new plate after 2 days without digesting or changing the medium". There is no need to add anti-CD3 and CD28 in the new plates.

Q#2. *What's the percentage of positive cells for Th0, Th1 and Th2 after one round experiment of this protocol?*

A. For WT control cells, Th0 cells cannot detect IL-4, but 10-15% express IFN-gamma, almost all Th1 cells express IFN-gamma, and 15-20% of Th2 cells express IL-4.

Q#3. *How many $CD4^{+}CD44^{lo}CD62L^{hi}$ T cells could you sort from one mouse (WT)? How many wells did you use for each condition (Th0/Th1/Th2)?*

A. About several million naive cells can be obtained from WT. Usually at least three wells for each condition so that the results can do statistical analysis.

Q#4. *How much the purity of naive T cells is important for this experiment?*

A. Using CD44 and CD62L antibodies to do the sorting and purity test for T cells should be good enough. The higher purity is, the better result is.

Q#5. *I used this protocol, however, my cells did not go through differentiation. After 7 days, I barely see less than 1% IFNgamma in Th1 and IL4 in Th2. Any suggestion?*

A. Maybe your intracellular staining does not work.

Q#6. *PMA from Sigma-Aldrich is not available in my country now, which another brand should I choose to buy this reagent?*

A. I haven't use the product from other companies, but I think PMA is not very special reagent, so the same reagent from a well-reputation company should work.

Q#7. *Can I start with pan T cells? Biolegend tech support saying that they start with pan T-cells. But some others told me that I need to start with naive T-cells. Which one is correct?*

A. I haven't work with pan T cells, but naïve T cells work well. Pan T cells include effector T cells as well as naïve T cells, so the purity of naïve T cells is not very high.

Q#8. *It looks that you do not need APCs (antigen presenting cells) in your culture system, why? What is the difference with or without APCs?*

A. The differentiation does not need peptide presented by APCs, and anti-CD3/CD28 can activate T cells. If you want to check the differentiation of peptide-specific T cell, you do need APCs instead of anti-CD3/CD28.

Q#9. *How many days plate coated with antiCD 3 and antiCD 28 is stable at 4 C before use?*

A. Coat with aCD3 one day before the experiment and keep at 4 degree.