ER-Hoxb8 neutrophil infection with Mycobacterium tuberculosis

Notes

- Grow ER-Hoxb8 neutrophil progenitors in media with 1 uM B-estradiol.
- I maintain them by splitting around 1:10 every 2 days in non TC-treated flasks.

Day -4: Estrogen withdrawal

- Spin down progenitors (5 min, 335 x g) and resuspend in 1x PBS to wash.
- Repeat for a total of 2 PBS washes.
- Resuspend after final spin in ER-Hoxb8 neutrophil media without estrogen and count.
- Plate in media without estrogen on non TC-treated flasks (or plates) at >500,000 cells/mL.
- Note: I try to split them to less than 1e6 cells/mL each day if they are undifferentiated, although they start to slow down in growth after several days of estrogen withdrawal.

- For *M. tuberculosis*, start a culture from frozen stock 4-5 days before infection in 7H9 media.

Day -2:

- Add media if cells are getting dense.
- Cells should start to adhere to the bottom of the flask.

Day -1: Harvest cells and re-plate for infection.

- Harvest floating fraction of cells and add cold PBS to bottom of flask.
- Incubate at 4C for 10-15 min, then pipette up and down to collect cells.
- Pool adherent fraction with suspension fraction and spin down.
- Resuspend in media and count.
- Re-plate cells at desired density for infection.
- Note: There are always some cells that never adhere to the bottom of the plate and many cells will be loosely adherent unless stimulated or infected.

Day 0: **Infect cells with *M. tuberculosis*.**

- Mtb cultures are washed in PBS to remove Tween 80 detergent (which will lyse mammalian cells).
- Clumps of bacteria are removed through sonication and a slow speed spin.
- Horse serum contains complement proteins that will aid in phagocytosis of Mtb.

1) Check OD of culture.
    a. OD600 of 0.2-1 (log phase growth) is ideal.
2) High speed spin—Spin down Mtb culture 5min at 2000 x g.
3) Resuspend in PBS to wash and spin down 5min at 2000 x g.
4) Resuspend in PBS and sonicate 3 x 30” with 15” pauses in between sonication.
5) Slow speed spin—Spin down 5 min at 40 x g.
6) Transfer cells in suspension to new tube and discard pelleted bacteria (clumps).
7) Measure ending OD of suspension.
8) Calculate how much bacteria to add to cells in a final concentration of 5% horse serum.
    a. OD600 of 1 = 3e8 CFU/mL.
9) Add bacteria to horse serum and add bacteria+horse serum to neutrophils at a final concentration of 5% horse serum in neutrophil media.
10) Phagocytosis should be complete by 4 hours post infection.
11) To avoid disrupting neutrophils, perform a half media change each day after infection by carefully removing half of the supernatant and adding fresh media on top.
12) Alternatively, a full media change can be done after the 4 hr phagocytosis period and every other day.

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