INTRODUCTION

A quiescent phenotype is observed naturally in HIV-exposed seronegative (HESN) commercial sex workers in Nairobi, Kenya, who have lower levels of CD4+ T cell activation but can otherwise elicit a normal immune response.

Previously, we found that low dose acetylsalicylic acid (ASA, brand name Aspirin (81 mg/day) reduced HIV target cells in the female genital tract. This revealed ASA's potential in preventing HIV.

OBJECTIVES & WORKFLOW

- Antibody Titration
  - Reduce background noise from non-specific staining
- Plate Staining Titration
  - Reduce background noise from non-specific staining
- CD3CD28 Stimulation Titration
  - Replace CD3/CD28 beads with pCD3+ and sCD28 to improve efficiency
  - Control ratio of pCD3 to pCD28

VolT Titration
- Determine ideal voltage before running experimental samples (data not shown)

METHODS

1. PBMCSstimulated with CD3/CD28 Dynabeads or pCD3+ and sCD28 for 72 hours
2. PBMCS were stained with intracellular and extracellular antibodies, and live/dead stain
3. PBMCS were fixed and permeabilized
4. Data collected on the LSRFortessa cytometer
5. Data analyzed using FlowJo, graphs prepared in Excel

RESULTS

Figure 5. Determining optimal antibody volumes for intracellular staining. Cells were single-stained with different concentrations of PmTOR or PS6k antibody. (A) Results from intracellular antibody titration. (B) Spread of intracellular marker expression. *Unstained.

Figure 6. Titration curves from extracellular plate staining titration. Master mix containing extracellular antibodies was titrated at different volumes. *Frequency of parent. *Histogram.

DISCUSSION

- 5 µl of PmTOR and 2 µl of PS6K were selected based off stain indices (Figure 5).
- 50 µl of intracellular master mix (Figure 6) and 75 µl of intracellular master mix (Figure 7) were selected based off titration curves.
- Condition 16 was selected for the low range and Condition 6 was selected for the high range pCD3 and sCD28 stimulation (Figure 8).
- Ideal voltages on the cytometer were selected to use during our study (data not shown).

CONCLUSION

Optimization ensures the reliability and reproducibility of the experiment and reduces day to day variation during experimentation.

We have optimized a panel for flow cytometry to determine ASA’s mechanism of inhibition on the mTOR pathway in CD4+ T cells.

SIGNIFICANCE

Determining ASA’s mechanism of inhibition on the mTOR pathway will provide more insight into how ASA induces a quiescent phenotype in the female genital tract.

REFERENCES


ACKNOWLEDGMENTS

- Fowke Lab Members
- Participants