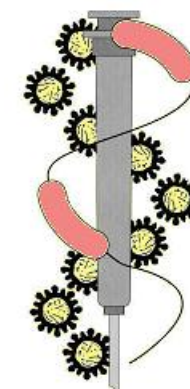


# Live oral typhoid vaccine Ty21a elicited cross-reactive multifunctional IL-17A producing T cell responses against *Salmonella enterica* serovars in humans.

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# Background

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Observations in field studies (M. Levine et al.) suggests that Ty21a partially cross-protects against *S. Paratyphi B* but not *S. Paratyphi A*.

Due to increases in the incidence of *S. Paratyphi A*, the development of an effective vaccine against *S. Paratyphi A* has become an priority.

Successful development of a vaccine against *S. Paratyphi A* will be aided by a better understanding of the complex human host-immune responses mediating protection against *Salmonella*.

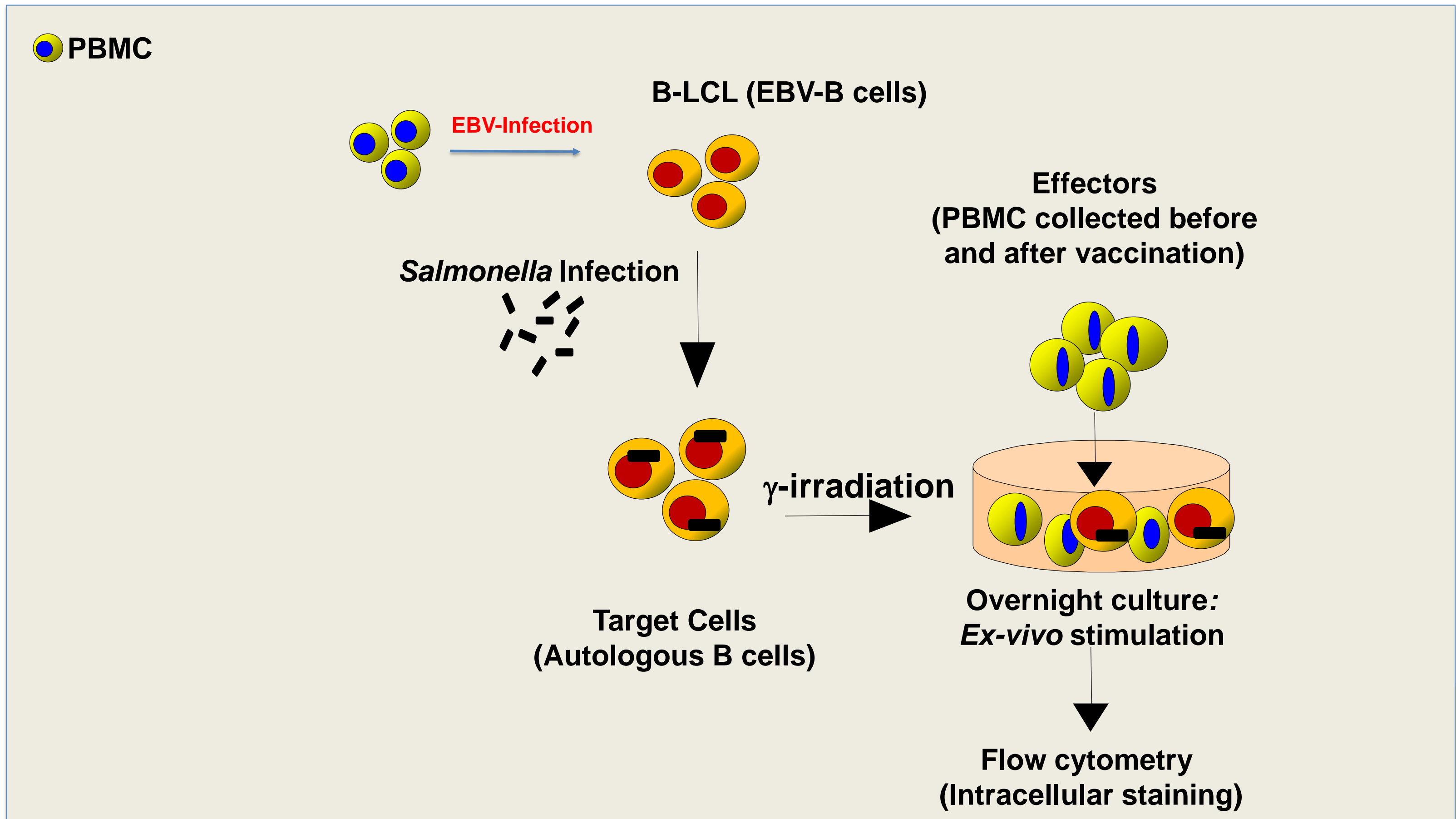
The exact protective immune mechanism of Ty21a in humans is not well understood. However, the vaccine induced robust Cell Mediated Immunity (CMI) mediated by CD8+ as well as CD4+ T cell responses are suggestive of its dominant role in protection.

Interleukin-17A producing (IL-17A+) T-cells are likely to play a critical role in protecting humans against intracellular organisms, including *Salmonella*-spp.

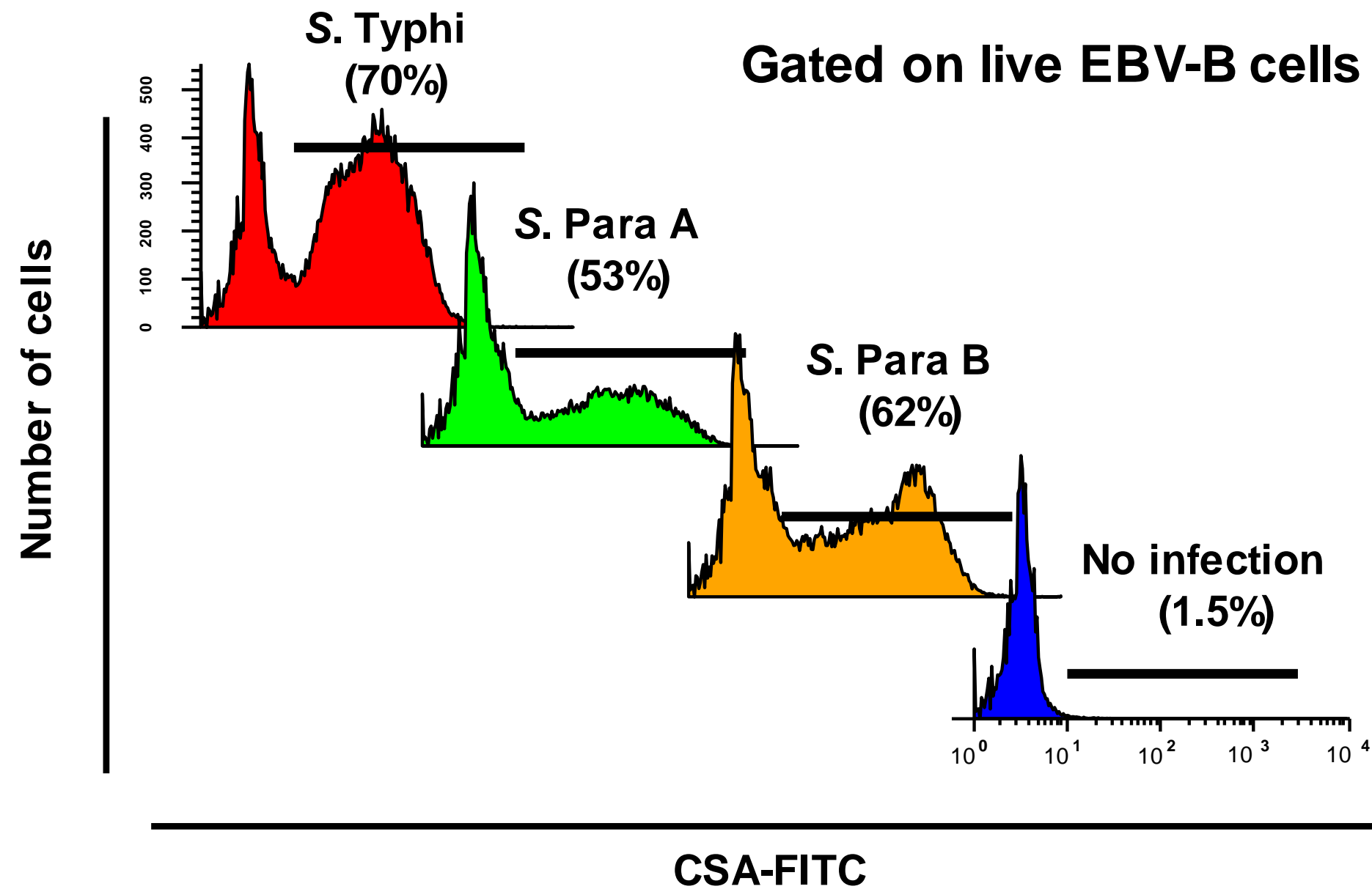
Therefore, we investigated the induction of *Salmonella* (*S. Typhi*, *S. Paratyphi A* and *B*)–responsive IL-17A+ CD4+ and CD8+ T-cells in volunteers immunized with the oral typhoid vaccine Ty21a.



Volunteers (n=8) were immunized with 3-4 doses of Ty21a and blood samples were drawn before (Day 0) and at several post-vaccination times (e.g., days 42 and 84). Purified PBMC were cryo-preserved.

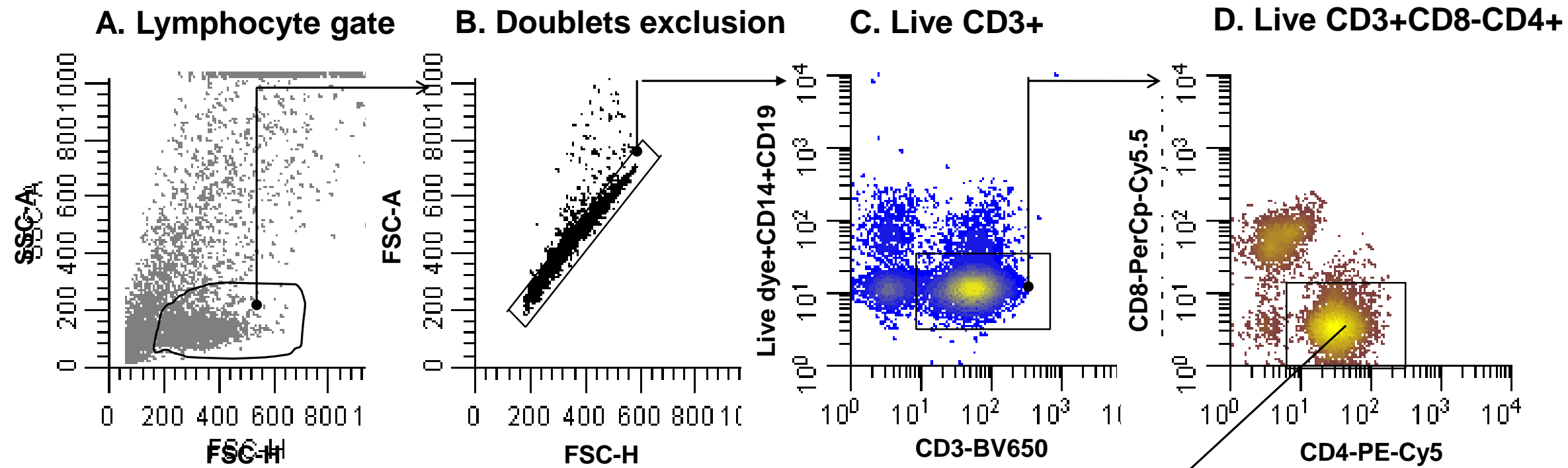


# Expression of *Salmonella*-antigens by infected EBV-B cell targets

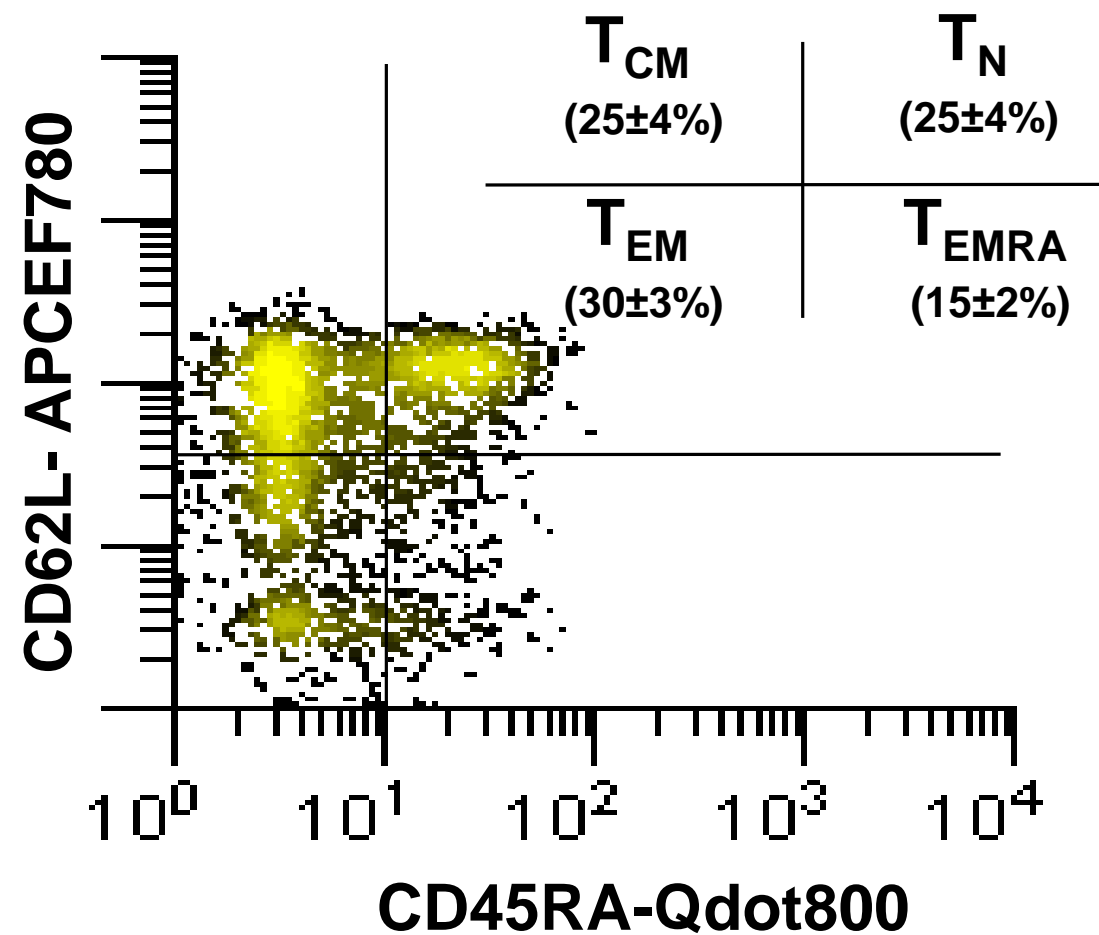


CSA-FITC: FITC conjugated anti-*Salmonella* antibody

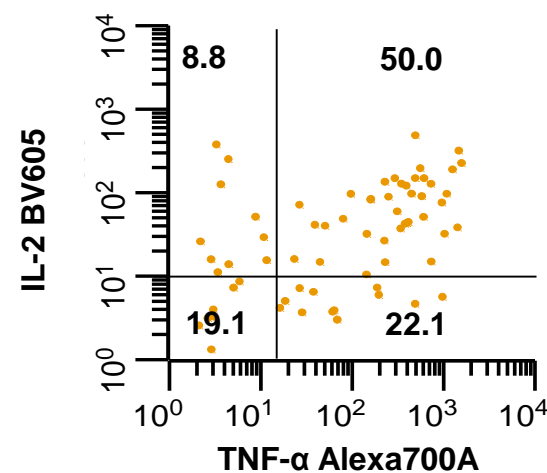
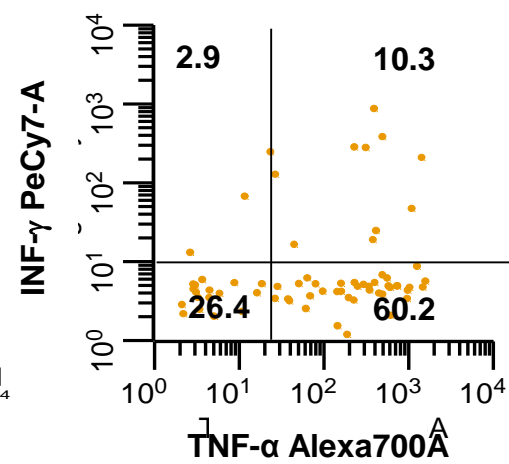
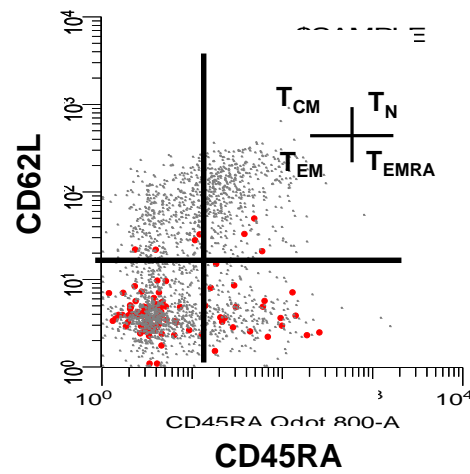
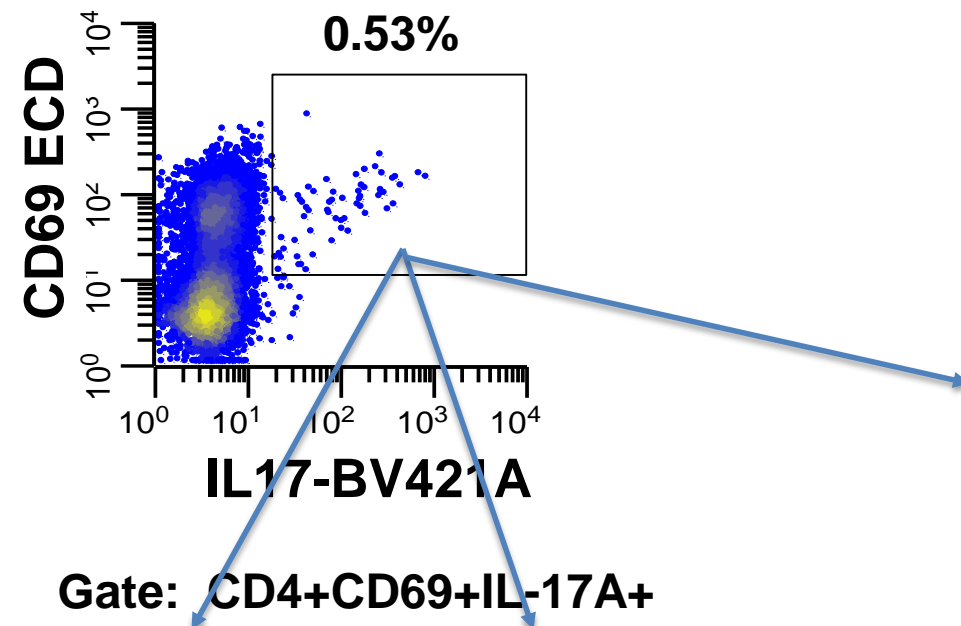




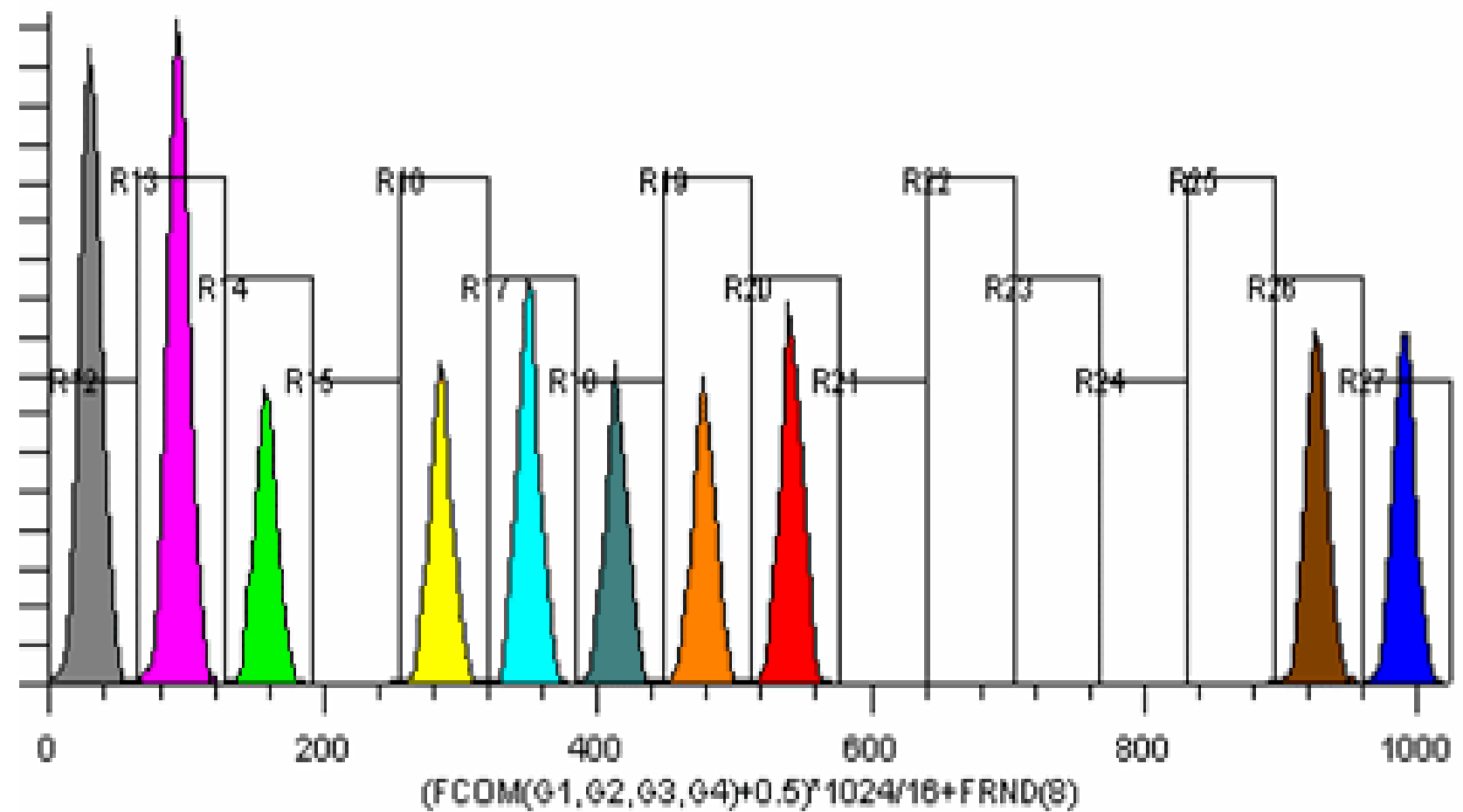
**E. CD4+ T cell memory**



Gate: Live CD3+CD8-CD4+  
(CD4+ T cells)



Functions measured: Interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-2, macrophage inflammatory protein-1 $\beta$  (MIP-1 $\beta$ ), IL-17A, and/or expression of cytotoxicity marker CD107a.



FCOM: Winlist 7.1: The tool classifies events based on combinations of selected gates

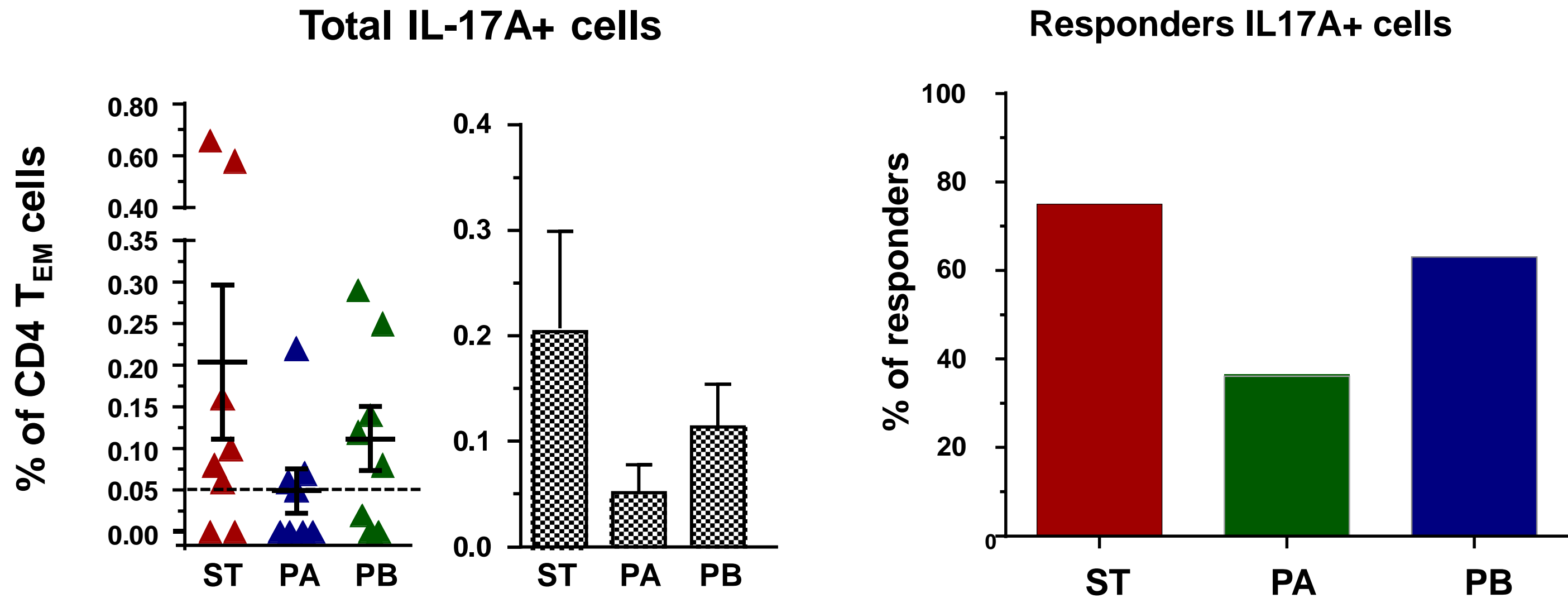
Representative volunteer #74:  
Post-vaccination day 42 PBMC stimulated with  
S. Typhi infected targets





## Results

# Post-vaccination increases in cross-reactive IL-17A producing CD4+ T<sub>EM</sub> cells following immunization with Ty21a



The peak-post vaccination increases (days 42 or 84) were calculated by subtracting the corresponding pre-vaccination (day 0) levels

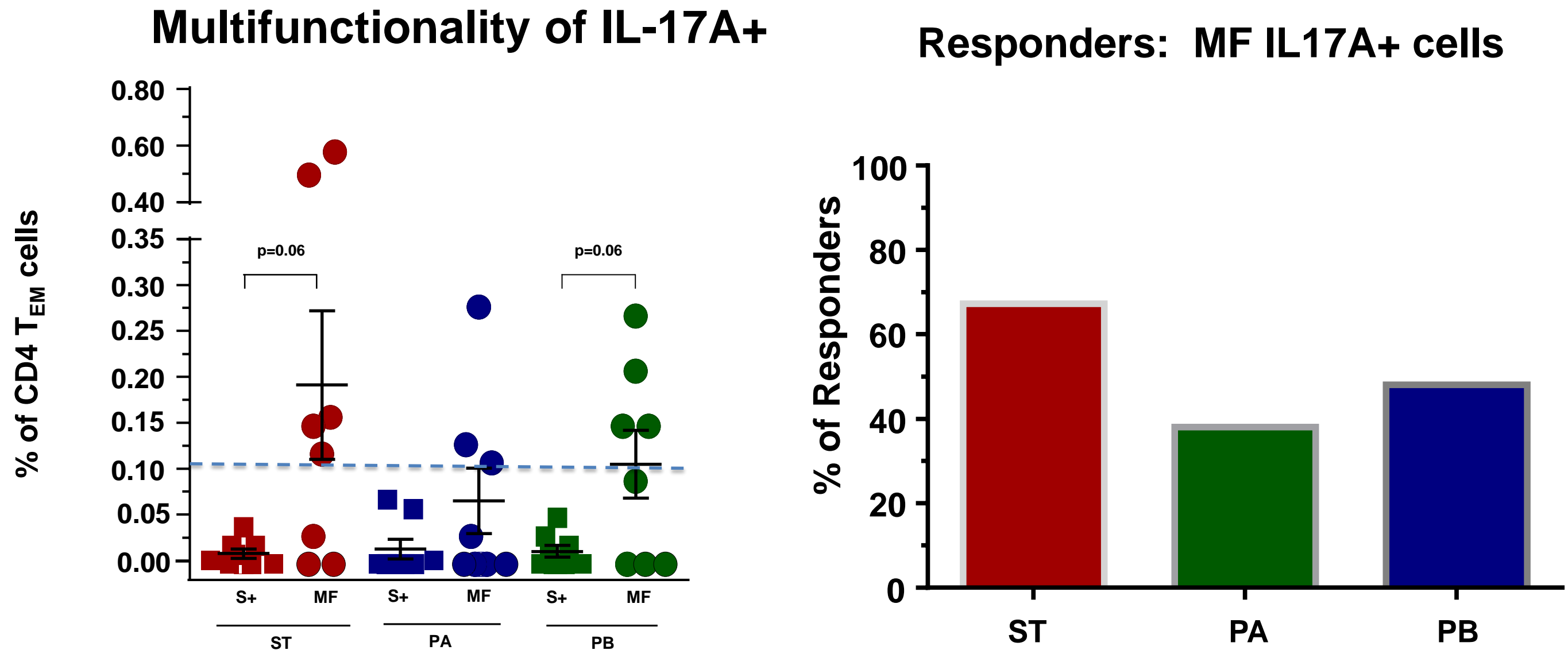
N=8

**ST:** S. Typhi-, **PA:** S. Paratyphi A-, **PB:** S. Paratyphi B-infected targets



## Results

# Post-vaccination increases in cross-reactive Multifunctional IL-17A+ producing CD4+ T<sub>EM</sub> cells



*Salmonella* specific IL-IL17A+ cells were segregated into S+ and MF cells. The peak-post vaccination increases (days 42 or 84) were calculated by subtracting the corresponding pre-vaccination (day 0) levels in each subset of cells.

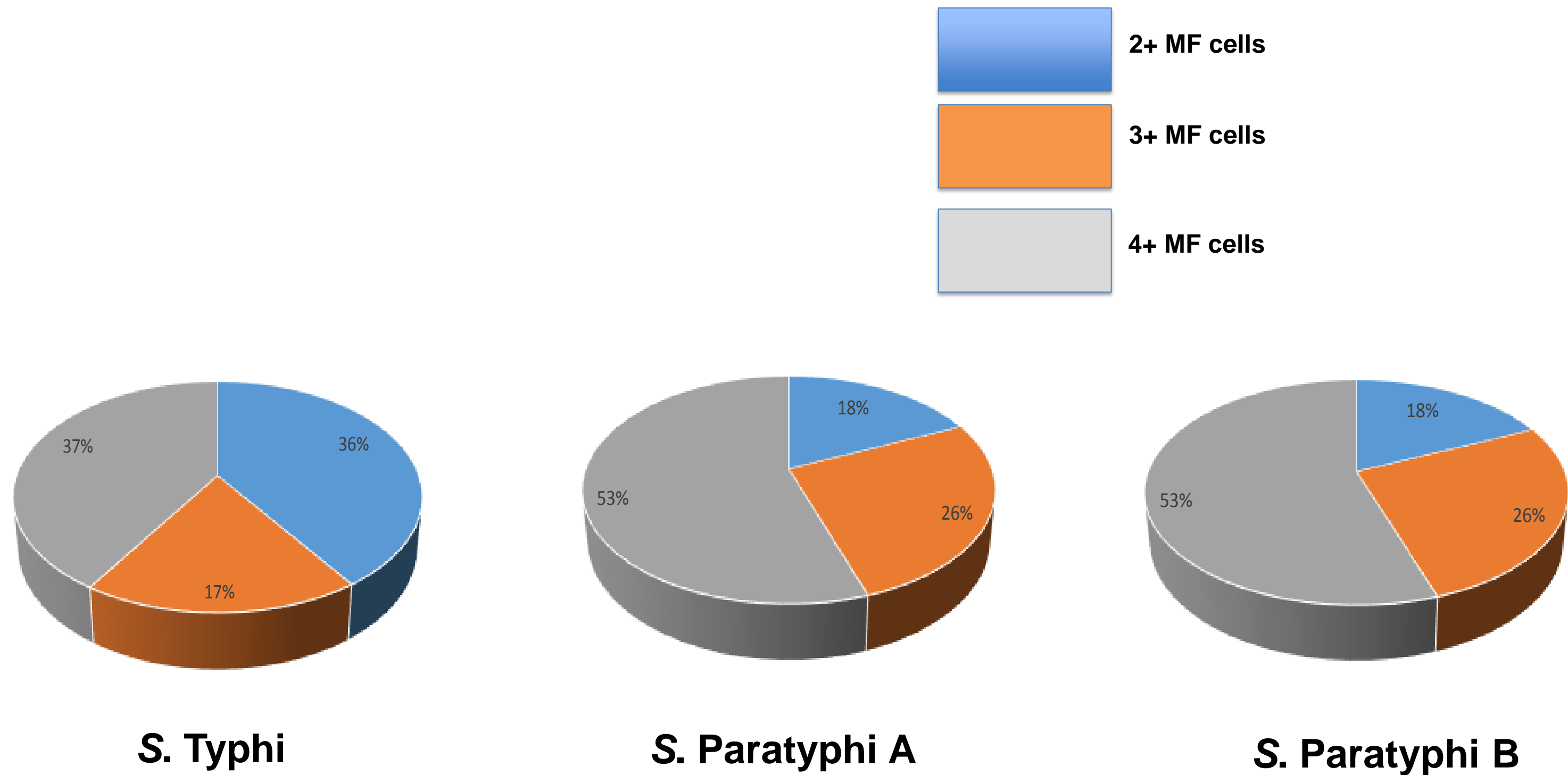
N=8

**ST:** *S. Typhi*-, **PA:** *S. Paratyphi A*-, **PB:** *S. Paratyphi B*-infected targets





# Characterization of Multifunctional IL-17A+ producing CD4+ T<sub>EM</sub> cells



# Summary

1. This is the first description of the induction of *Salmonella* cross-reactive IL-17A cells in CD4+ T cell subsets with their effector/memory and Multifunctional characteristics.
2. Post-vaccination increases in IL-17A producing CD4+ T effector-memory ( $T_{EM}$ ; CD45RA-CD62L-) following stimulation with *S. Typhi*-infected targets were observed in 75% of the volunteers studied (n=8). Similar cross-reactive responses against *S. Paratyphi* A- and B-infected targets were observed in 38% and 63% of volunteers, respectively.
3. *S. Typhi*- and *S. Paratyphi* B- but not *S. Paratyphi* A-responsive MF IL-17A+ CD4+  $T_{EM}$  cells showed a strong trend to be higher than the corresponding Single+ cells.
4. *Salmonella*-responsive IL17A+ CD4+  $T_{EM}$  cells were 65-70% MF producing three or more (3+ or 4+) cytokines or expressing CD107a.
5. In contrast, *Salmonella*-infected target-responsive  $T_{EM}$  or RA positive  $T_{EM}$  ( $T_{EMRA}$ : CD45+CD62L-) CD8+ T cell subsets from 38% of the same volunteers showed similar increases of IL17A+ MF cells (data not shown today)



# Overall Conclusions

- Immunization with Ty21a induces predominantly CD4+ MF Th17 helper cells, which might be an important component of vaccine induced protective CMI responses.
- These observations detailing vaccine elicited immune responses in humans are likely to contribute to advance the development of more effective vaccines against *S. Typhi*, novel vaccines against *S. Paratyphi A* and *B* infection, as well as bivalent vaccines.



## Acknowledgements

**Marcelo B Sztejn**

### **CVD Immunology Group**

Regina Harley  
Cathy Storrer

Haiyan Chen  
Shah Jafar

Kirsten Lyke  
Rosangela Mezghanni  
Monica McArthur  
Franklin Toapanta  
Jayaum Booth  
Stephanie Fresnay  
Rekha Rapaka  
Paula Bernal  
Mark Rudolph

Glen Hatfield  
Kaushiki Mazumdar  
Ming Bell  
Jeffrey Floyd  
Vidhya Prasad

**Myron M Levine**

Robin Barnes  
Volunteer recruiting center staff

**Funding:** NIAID, NIH, DHHS grants R01-AI036525, U19 AI082655 (Cooperative Center for Translational Research in Human Immunology and Biodefense [CCHI]), and U54-AI057168 (Regional Center for Excellence for Biodefense and Emerging Infectious Diseases Research Mid-Atlantic Region [MARCE] and U19-AI109776 (Center of Excellence for Translational Research [CETR]).

