Recent advances on the complex and multifaceted T memory and effector immunity elicited to *S.* Typhi in humans

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8th International Conference on
Typhoid Fever and Other Invasive Salmonelloses

Dhaka, Bangladesh – March 1, 2013

Lack of known immunological correlates of protection in typhoid fever

- Ab to *S.* Typhi antigens (e.g., Vi, LPS O) are likely to play an important role in defense against typhoid bacilli when they are extracellular.
- In contrast, since *S.* Typhi persists intracellularly, thereby avoiding destruction by Ab and C', CMI is expected to be essential in eliminating *S.* Typhi from the infected cells.
- Both adaptive immune mechanisms (CMI & Ab) are expected to provide critical support to innate immunity in the mucosal microenvironment and elsewhere

Effector Ab responses to *S.* Typhi

1. Serum IgG and IgA Abs to *S.* Typhi antigens
   - LPS O, flagella, Vi, OMPs, hsp, others? (Elisa)
   - Opsonophagocytosis, ADCC, bactericidal (Functional)
2. IgG and IgA ASC to *S.* Typhi antigens in circulation
   - LPS O, flagella, Vi, OMPs, others?
   - 7-10 days after immunization (longevity in the gut and other tissues unknown)
3. Anti-*S.* Typhi specific secretory IgA (SIgA) in intestinal lavage fluids and stools of subjects exposed to *S.* Typhi
4. B memory cells (e.g., LPS, Vi)

Immunological correlates of protection in typhoid fever: Ab summary

It is not known whether Ab to common S. Typhi antigens, particularly to O, H and Vi, particulalry those with defined functional activities, actually

(1) Mediate protection,

(2) Act in conjunction with other innate and adaptive responses or

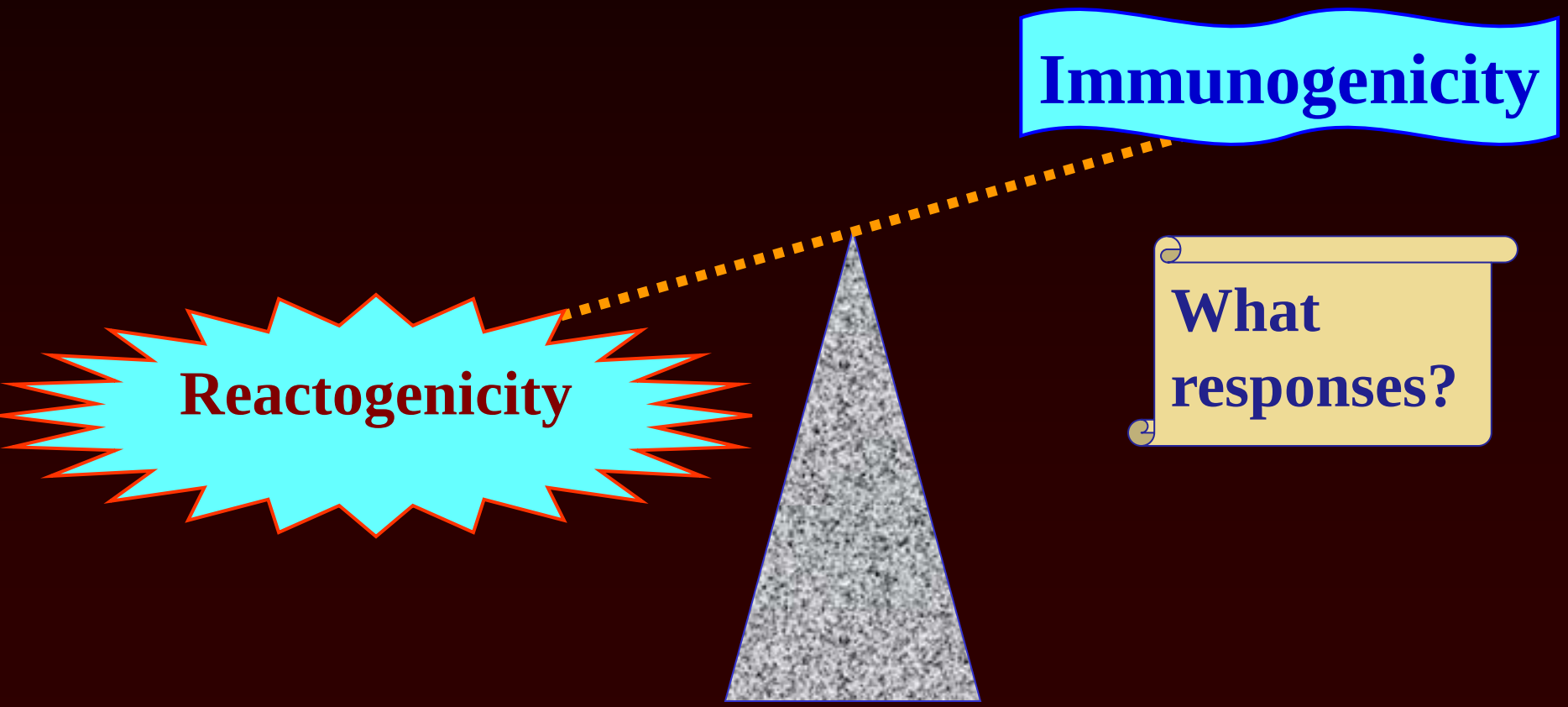
(3) Serve as a surrogate for the presence of other more dominant protective immune responses (e.g., CMI) that will eventually lead to the elimination of this intracellular bacteria from the host

Immunological correlates of protection in typhoid fever: Hypothesis

Both CMI and Ab responses play central roles in protection in typhoid fever and are elicited following immunization with attenuated *S.* Typhi vaccine candidates

Vaccine Development

The never ending search for the optimal balance



Attenuated *S.* Typhi vaccine strains

Organism	Strain	Mutations	Metabolic Pathways
S. Typhi	CVD 908	Δ*aroC* Δ*aroD*	Biosynthesis of aromatic aa
S. Typhi	CVD 908-*htrA*	Δ*aroC* Δ*aroD* Δ*htrA*	Biosynthesis of aromatic aa heat-shock protein
S. Typhi	CVD 909	Δ*aroC* Δ*aroD* Δ*htrA, viaB*	Biosynthesis of aromatic aa heat-shock protein Constitutively expresses Vi
S. Typhi	Ty21a	Chemical mutagenesis	Many

Key Effector CMI to *S.* Typhi in orally immunized subjects (1)

- Effector responses to *S.* Typhi-infected targets:
  - **Cytotoxic T lymphocytes (CTL) activity** (^{51}Cr-release assays; granzyme; CD107 staining by flow cytometry)
  - **IFNγ production** (TNF-α, others)
  - **Mediated by both CD8$^+$ (dominant) and CD4$^+$ cells**
  - **CD8+ CTL activity restricted by:**
    - Classical class Ia molecules (HLA-A, B, C)
    - Non-classical class-Ib molecules (HLA-E)

Key Effector CMI to *S.* Typhi in orally immunized subjects (2)

- **Proliferation** and predominant **type-1 cytokine responses** to soluble *S.* Typhi antigens (e.g., flagella)
  - IFNγ, TNFα, IL-10 in the absence of IL-4, IL-5 & IL-6
  - IFNγ produced predominantly by $CD4^+$ cells
- **Homing to mucosal and non-mucosal tissues:** IFN-γ production by **central** and **effector memory T** subsets that express, or not, the gut homing molecule integrin α_4/β_7
- Presence of **long-term multifunctional** HLA-E-restricted $CD8^+$ cells co-expressing **IFN-γ, TNF-α** and **CD107**

Detailed CMI studies to S. Typhi-infected autologous cells in Ty21 vaccinees

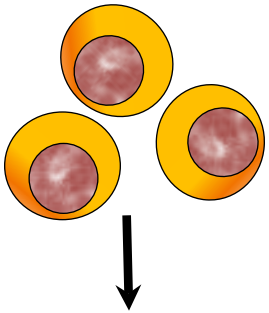
1. Define the kinetics of CMI to *S.* Typhi-infected autologous cells elicited by Ty21a in humans
2. Study **IL-17A** responses elicited in Ty21a vaccinees
   - IL-17A is a pro-inflammatory cytokine produced by CD4+ and CD8+ T cells. Recently shown to play a key role in mucosal immunity
   - Measure the multi-functionality of T cell responses following Ty21a immunization

CMI to *S.* Typhi-infected autologous cells in Ty21 vaccinees: Multi-functional T cells

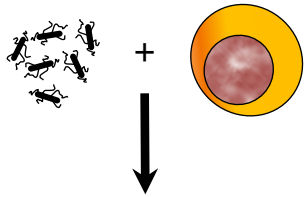
- **Why study multi-functionality of the T cell responses following Ty21a immunization?**
  - Studies have shown that multifunctional T cells, those producing 2 or more cytokines simultaneously, might be critical effectors in protection from infection in animals and humans (e.g., HIV, Mtb)
  - Technological advances and unsupervised flow ctyometry analysis packages enable the study of all possible combinations of many cytokines to define multi-functional CD8+ T cell subsets

Experimental Design: In vitro stimulation with *S.* Typhi-infected targets

Target Cells
Autologous EBV-LCL, 721.221.AEH, blasts

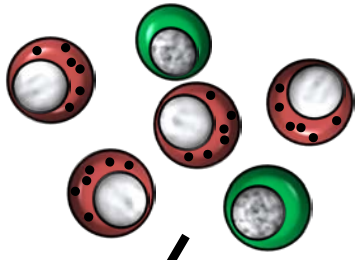


S. Typhi infection



γ-irradiation

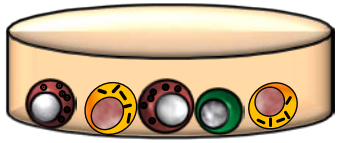
Effector cells
Ex vivo PBMC collected before and 1, 2, 4, 8, 10, 14, 21, 28, 60, 90, 180, 360 days after Ty21a immunizaiton



CD8+ T effectors
(CTL, cytokines)

CD4+ T effectors
(cytokines)

Others?



14-18 hrs

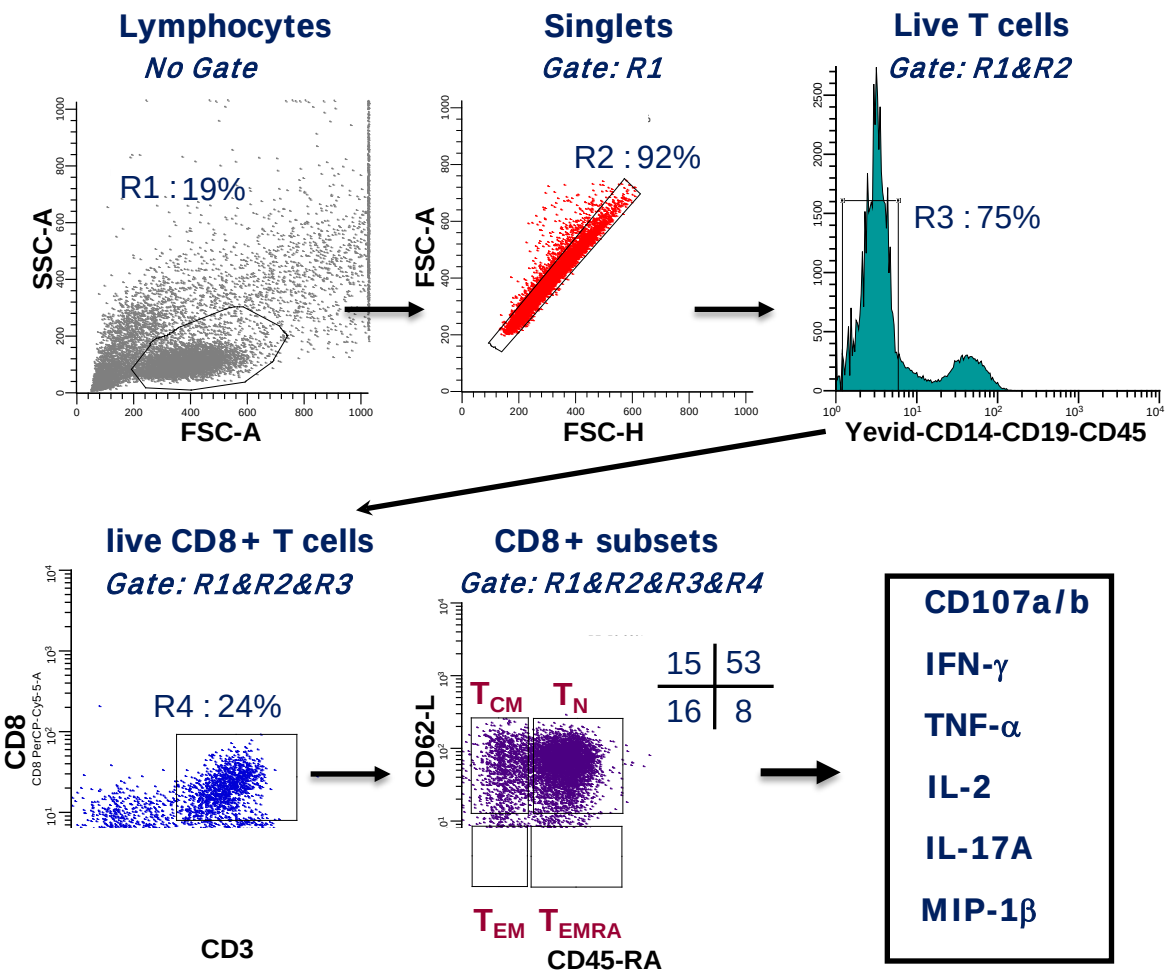
Flow cytometry
(Tm subsets, CD107a, IFN-γ, TNF-α, MIP-1β, IL-17A, IL-2, etc)

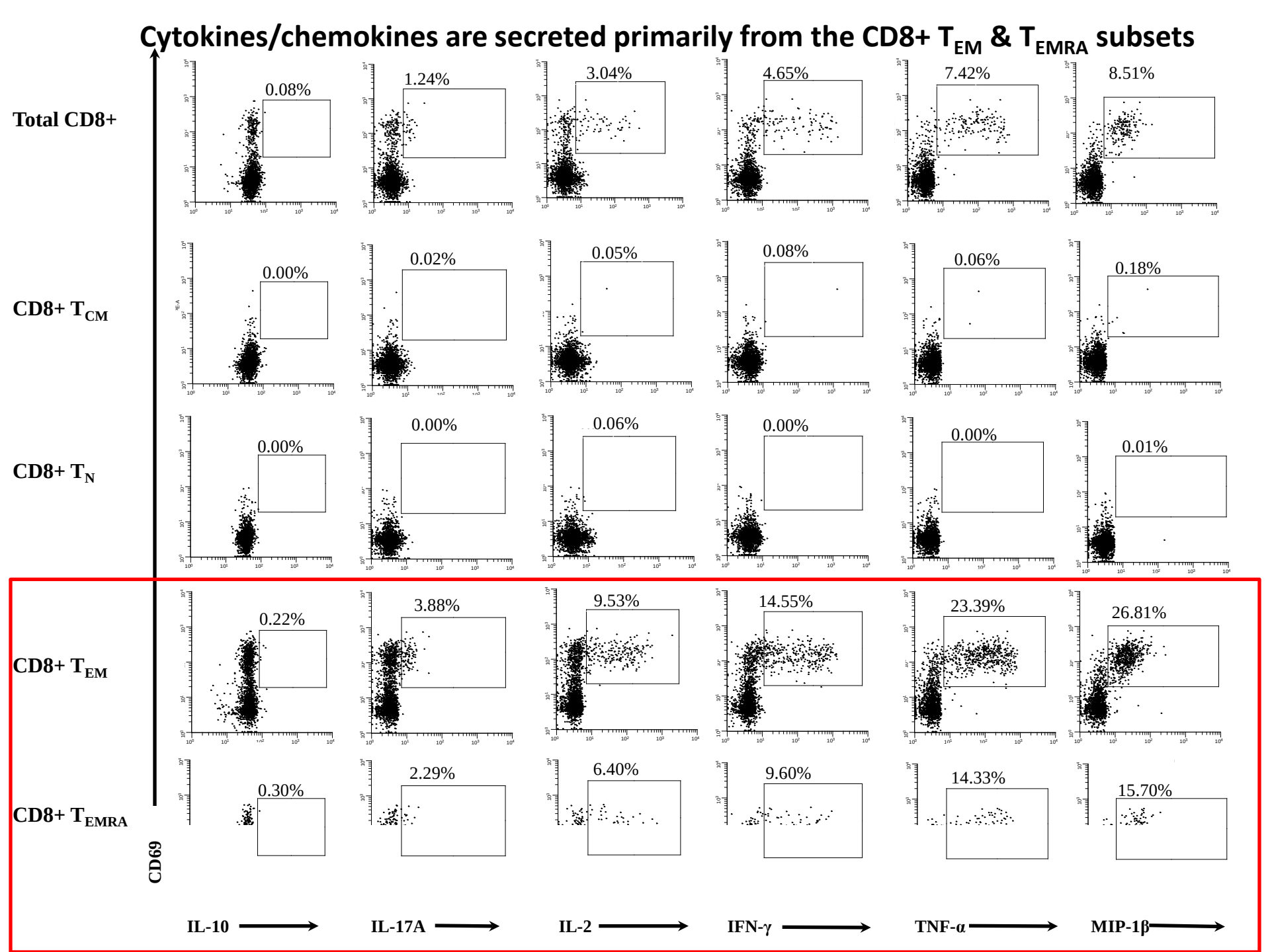
Experimental Design: Flow cytometry gating

TABLE 1. Cell phenotypes

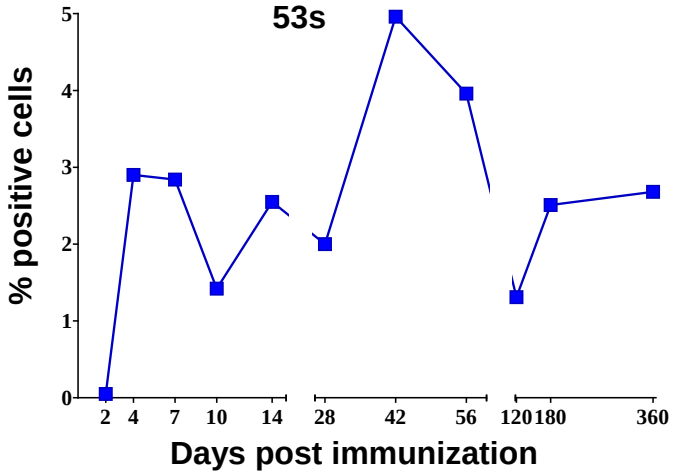
Phenotype	Marker
"Dump" channel	
Dead cells	Yevid
Monocytes	CD14
B cells	CD19
EBV-LCL	CD45
T cell subset	
Naïve	$CD45RA^{+}CD62L^{+}$
Central memory (T_{CM})	$CD45RA^{-}CD62L^{+}$
Effector memory (T_{EM})	$CD45RA^{-}CD62L^{-}$
Effector memory (T_{EMRA})	$CD45RA^{+}CD62L^{-}$
Degranulation (CTL)	
LAMP-1	CD107a
Cytokines/chemokines	
	IFN-γ
	TNF-α
	IL-2
	IL-17A
	MIP-1β

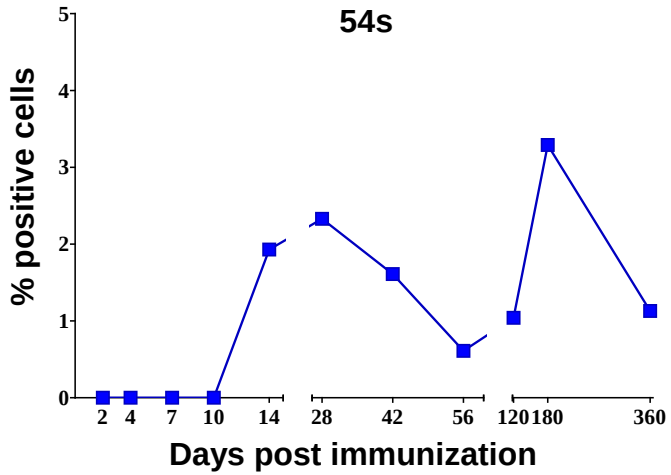
Sequential gating strategy

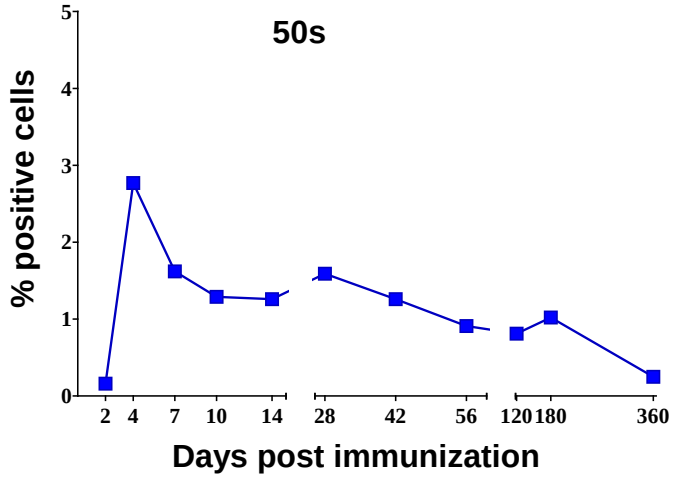



Cytokines/chemokines are secreted primarily from the CD8+ T_{EM} & T_{EMRA} subsets
Total CD8+
CD8+ T_{CM}
CD8+ T_N
CD8+ T_{EM}
CD8+ T_{EMRA}
CD69
0.08%
1.24%
3.04%
4.65%
7.42%
8.51%
0.00%
0.02%
0.05%
0.08%
0.06%
0.18%
0.00%
0.00%
0.06%
0.00%
0.00%
0.01%
0.22%
3.88%
9.53%
14.55%
23.39%
26.81%
0.30%
2.29%
6.40%
9.60%
14.33%
15.70%
IL-10
IL-17A
IL-2
IFN-γ
TNF-α
MIP-1β

IL-17A is secreted by CD8+ T_{EM} cells in response to Ty21a immunization in humans

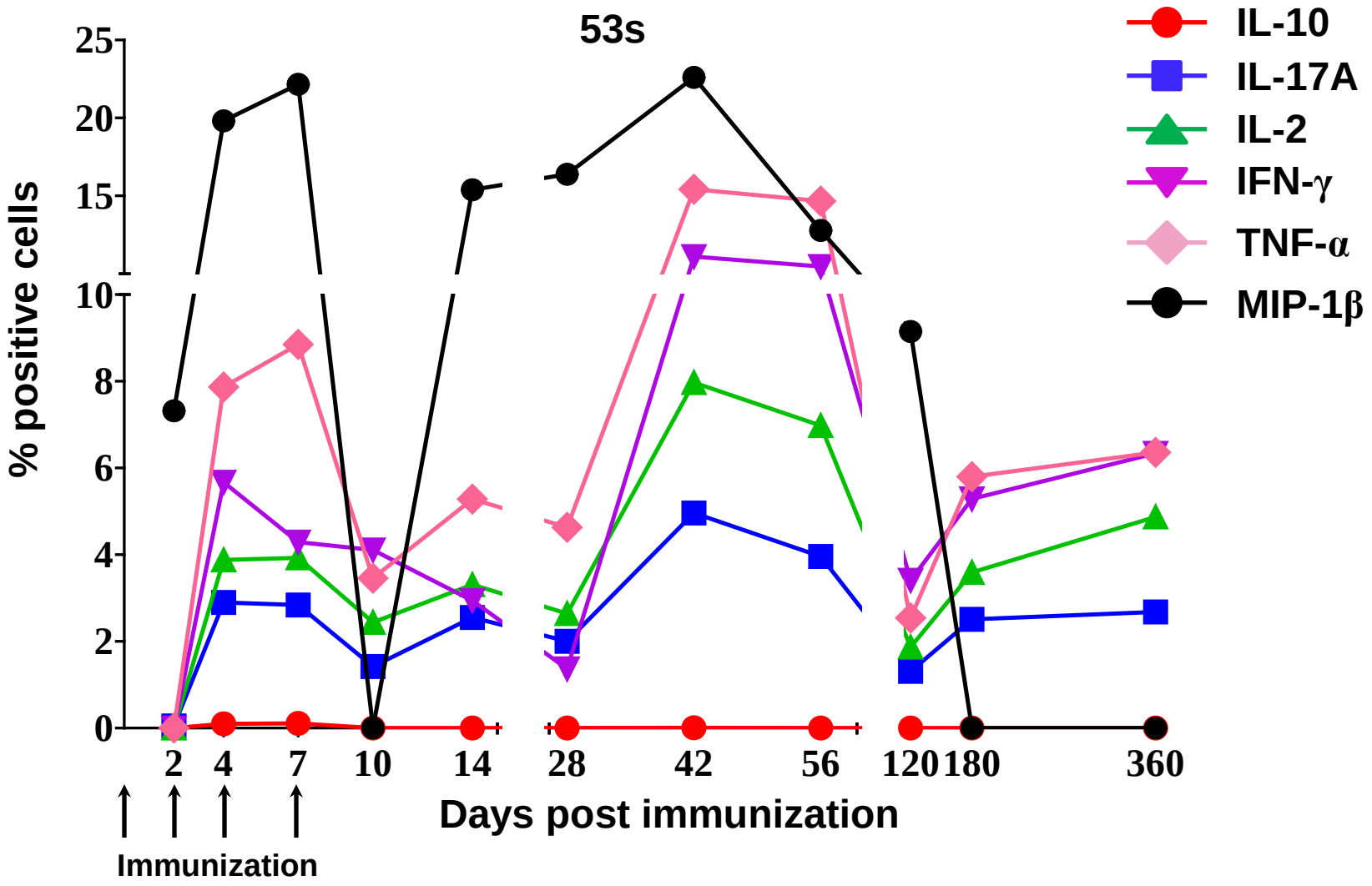




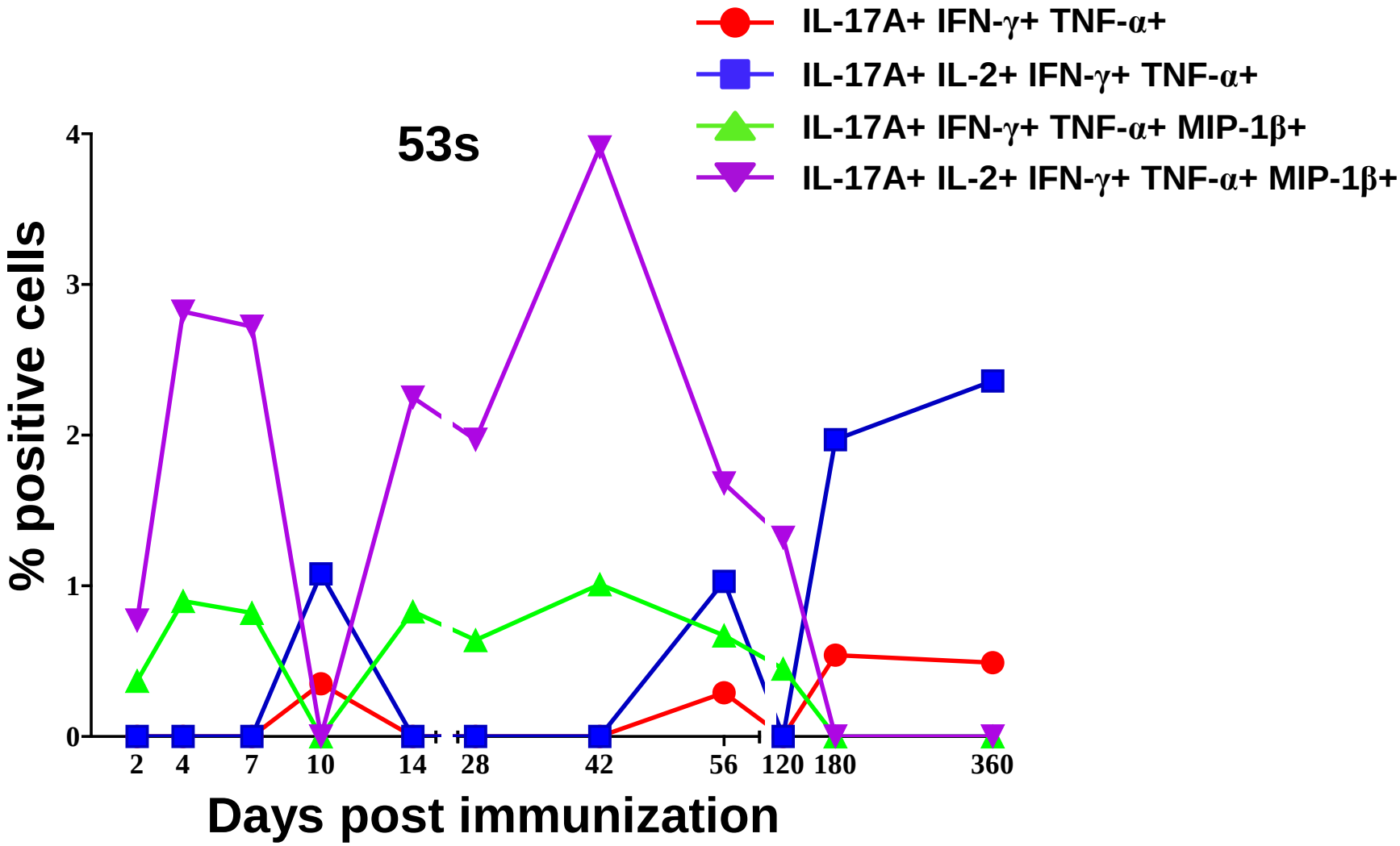


IL-17A

Kinetics of net CD8+ T_{EM} responses to stimulation with *S.* Typhi-infected autologous cells in Ty21a vaccinees



IL-17A+ multifunctional CD8+ T_{EM} following exposure to *S.* Typhi-infected autologous cells



Conclusions (I)

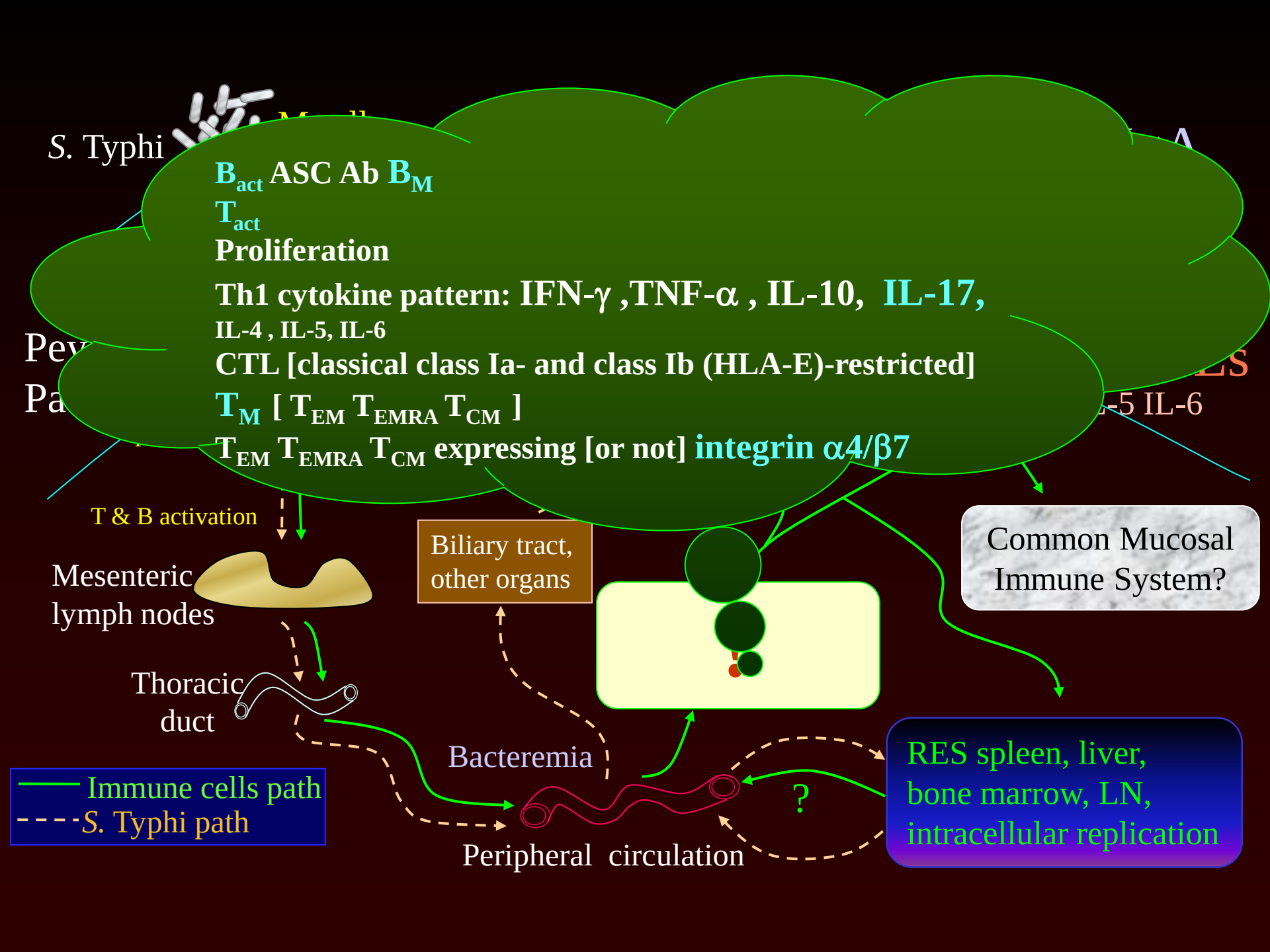
- Ty21a-immunized volunteers exhibited CD8+ T_{EM} and T_{EMRA} cell responses following stimulation with *S.* Typhi-infected autologous EBV-LCL.

- Multifunctional CD8+ T cells are present in response to Ty21a immunization.

  - Multifunctional T cells have been reported to correlate with lack of disease progression in HIV, have been identified as a potential correlate of protection for *Leishmania major* immunization in a mouse model, etc.

Conclusions (II)

- This is the first demonstration of IL-17A production in response to Ty21a immunization
  - IL-17A secreting cells have been shown to play a role in mucosal immunity
- IL-17A is secreted from **multifunctional** cells that co-produce IL-2, IFN-γ, TNF-α, and/or MIP-1β
- There is heterogeneity in the kinetics of the immune responses to Ty21a immunization.
  - **Bi-phasic & tri-phasic** responses to *S.* Typhi-infected autologous EBV-LCL are typical. Thus, evaluating a single time point may fail to accurately evaluate responsiveness


S. Typhi
B_{act} ASC Ab B_M
T_{act}
Proliferation
Th1 cytokine pattern: IFN-γ ,TNF-α , IL-10, IL-17,
IL-4 , IL-5, IL-6
CTL [classical class Ia- and class Ib (HLA-E)-restricted]
T_M [T_{EM} T_{EMRA} T_{CM}]
T_{EM} T_{EMRA} T_{CM} expressing [or not] integrin α4/β7
T & B activation
Mesenteric lymph nodes
Thoracic duct
Biliary tract, other organs
Common Mucosal Immune System?
Bacteremia
Peripheral circulation
RES spleen, liver, bone marrow, LN, intracellular replication
?
Immune cells path
S. Typhi path

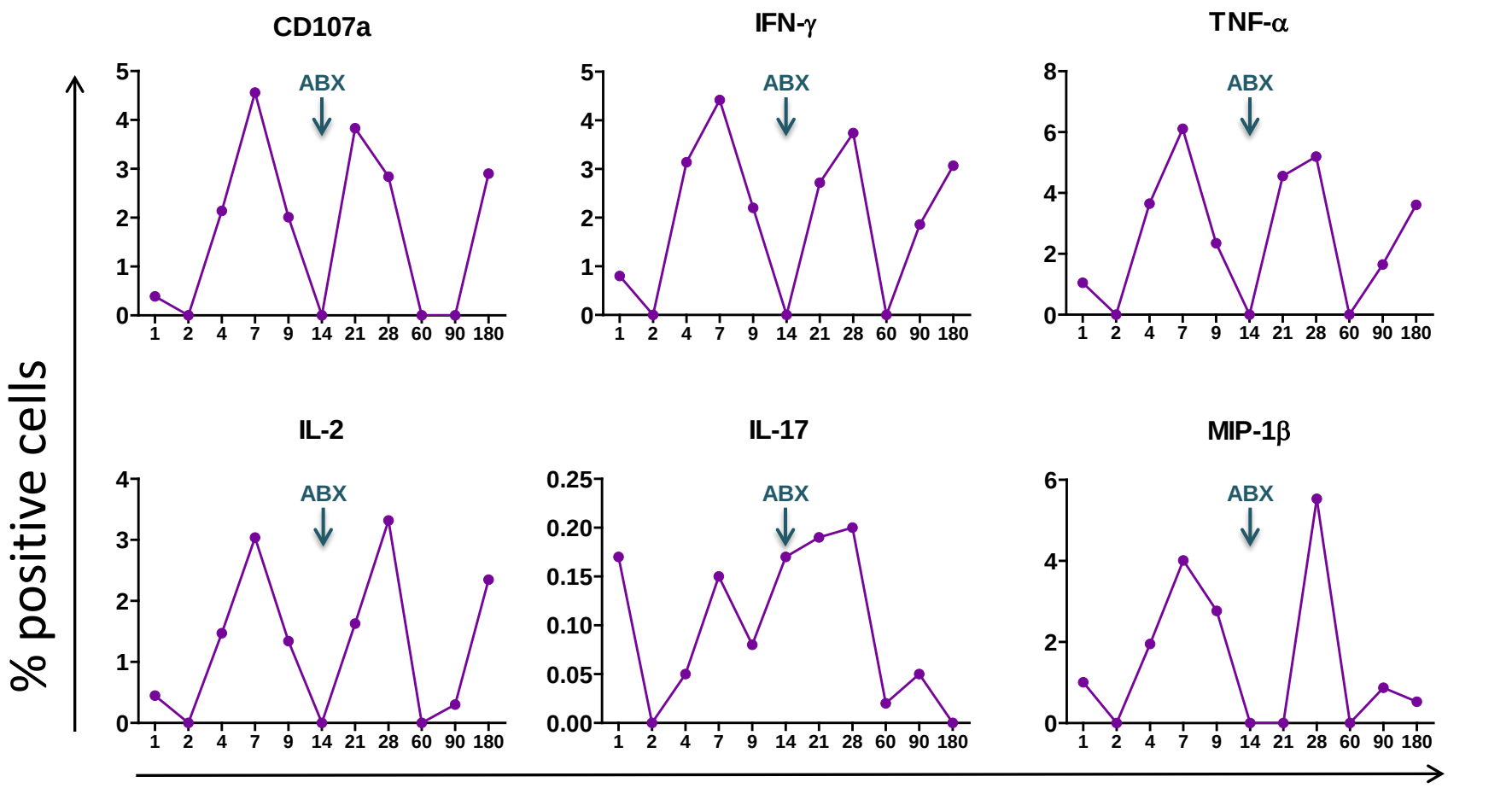
The question remains:

Which of these complex and heterogeneous CMI responses, if any, are associated with protection from typhoid fever?

To answer this question, and to better understand typhoid disease, we recently initiated a collaboration with Dr. Pollard and his team at Oxford who have re-established a human challenge model with wild-type *S.* Typhi

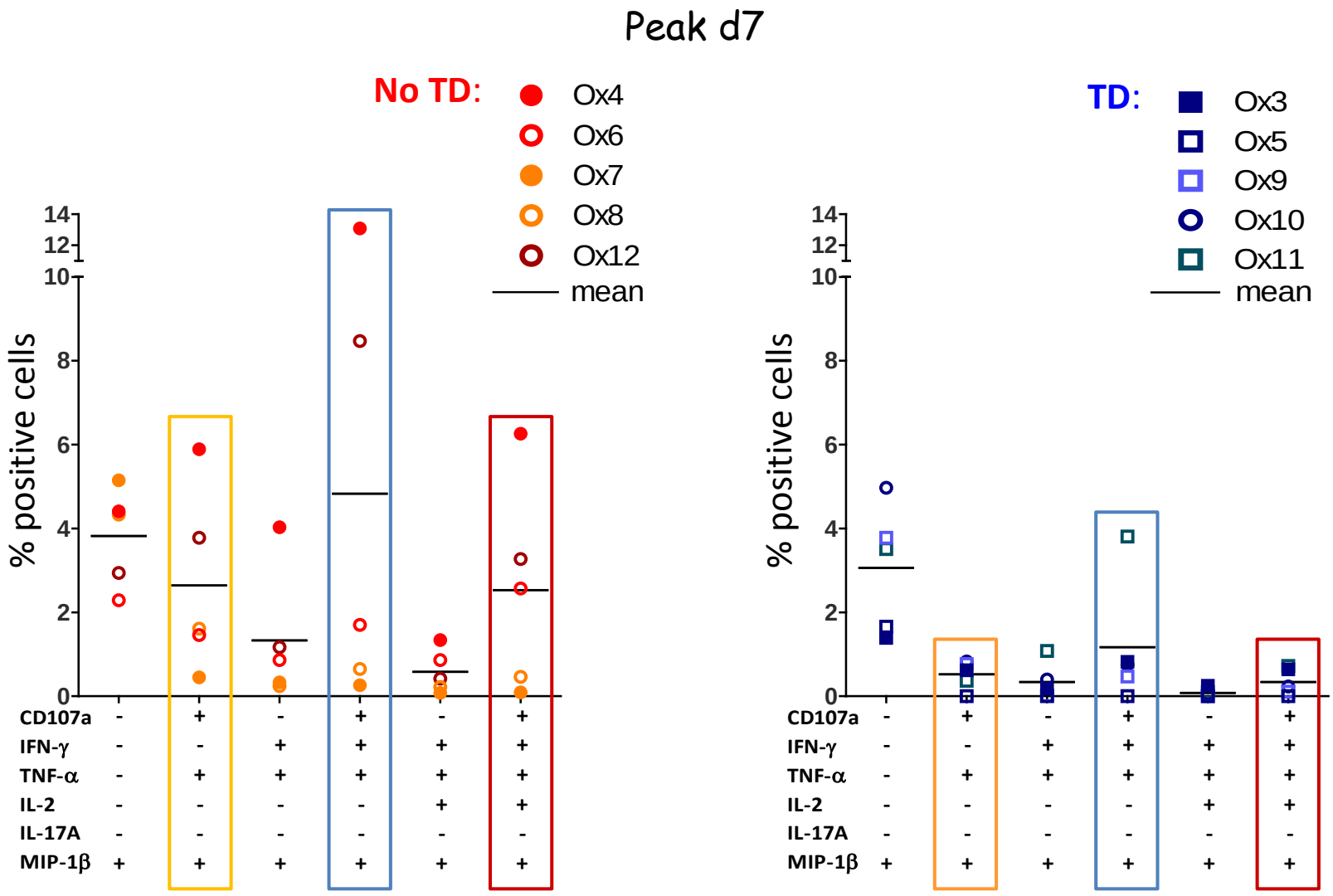
Multiphasic kinetics of CMI responses by CD8+ T_{EM} to *S.* Typhi-infected AEH-cells (HLA-E-restriction)

This volunteer (Ox6) did not develop typhoid fever (No TD)



ABX : antibiotics

Multifunctional CD8+ T_{EM} to *S.* Typhi-inf B-LCL in wild-type *S.* Typhi-challenged subjects



Mass cytometry

Mass cytometry (CyTOF), a transformational flow cytometry technology to measure human immune responses by simultaneously evaluating >35 parameters/cell

S. Typhi appears to be such an effective pathogen, at least in part, by being exquisitely stealth.

Thus, identifying the effective immunological CoP among a sea of non-protective or downregulatory immunity might hold the key for the development of more effective vaccines.

Identification of the precise immunological correlates of protection (either mechanistic or non-mechanistic), can significantly advance the development of broad spectrum vaccines for enteric fevers (e.g., *S.* Paratyphi A, *S.* Paratyphi B)

Acknowledgements

Center Vaccine Development, UMB

Immunology Group

Monica McArthur

Stephanie Fresnay

Franklin Toapanta

Rosangela Mezghanni

Rezwan Wahid

Cathy Storrer

Regina Harley

Clinical studies

Myron Levine

Robin Barnes

Oxford Biomedical Research Centre

Oxford Team

Andrew J Pollard

Claire Jones

Claire Waddington

Thomas Darton

Christoph Blohmke

Gordon Dougan

Jeremy Farrar

Paul Langford

Brian Angus

Derrick Crook

Stephen Lockhart

Supported by grants U19 AI082655 (UMB-CCHI) and R01 AI-36525