



Immune response and correlates of protection against typhoid

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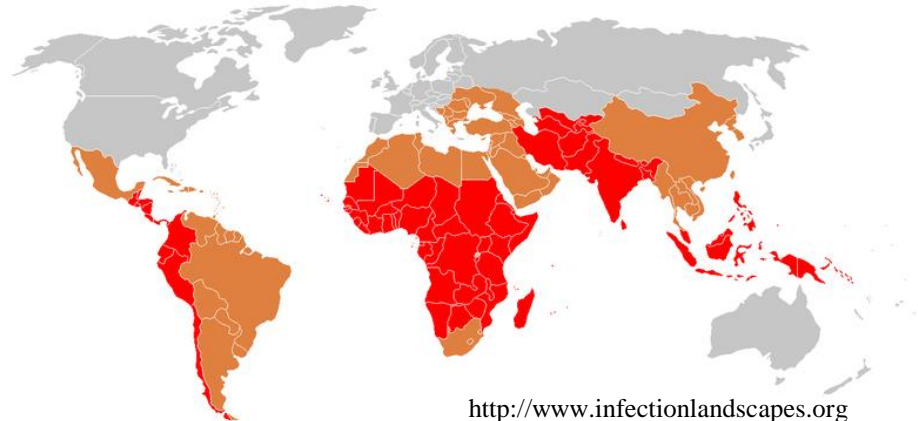
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Correlates of enteric vaccine-induced protection
Les Pensières - Fondation Mérieux

March 22, 2016 - Annecy - France

Salmonella enterica serovar Typhi

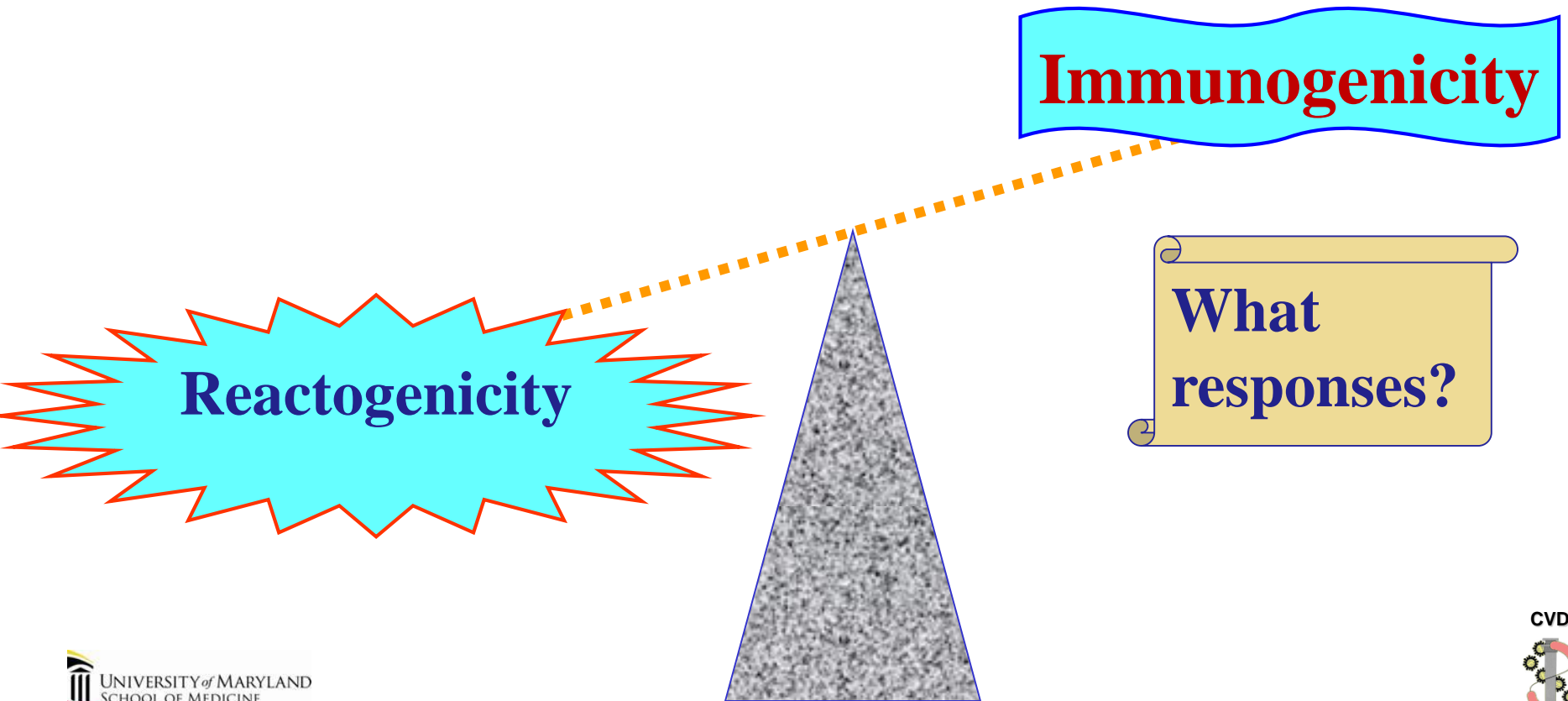
- It is a human restricted **intracellular** Gram-negative bacterium that infects both phagocytic and non-phagocytic cells
- The causative agent of typhoid fever
- >20 million of individuals infected annually
- >200,000 deaths/year
- Appearance of antibiotic resistance in Asia and northeast Africa



<http://www.infectionlandscapes.org>

Vaccine Development

The never ending search for the optimal balance



Identification of the precise immunological correlates of protection (either mechanistic or non-mechanistic), can:

1. Define the effector immunity to be pursued during vaccine development
2. Help predict long-term protection
3. Accelerate the development of broad spectrum vaccines for enteric fevers (e.g., *S. Paratyphi A*, *S. Paratyphi B*)
4. Advance the development of live vector vaccines

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

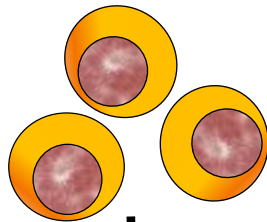
Immunological correlates of protection in typhoid fever: Ab summary

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

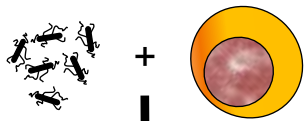
- (1) Mediate protection,
- (2) Act in conjunction with other innate and adaptive responses or
- (3) Serve as a surrogate for the presence of other more dominant protective immune responses (e.g., CMI) that will eventually lead to the elimination of this intracellular bacteria from the host

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

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



S. Typhi infection

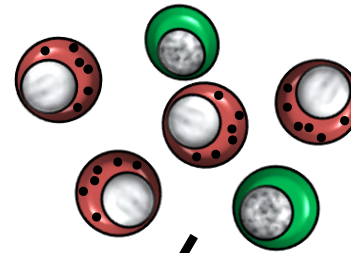


γ -irradiation



Effector cells

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



CD8+ T effectors
(CTL, cytokines)

CD4+ T effectors
(cytokines)

MAIT

**APC's, B cells,
Others?**

ELISpot (IFN- γ)

14-18 hrs

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

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

- ❖ Effector responses to *S. Typhi*-infected targets:
 - **Cytotoxic T lymphocytes (CTL) activity** (⁵¹Cr-release assays; granzyme; CD107 staining by flow cytometry)
 - **IFN γ 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)
 - IFN γ , TNF α , IL-10 in the absence of IL-4, IL-5 & IL-6
 - IFN γ 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**

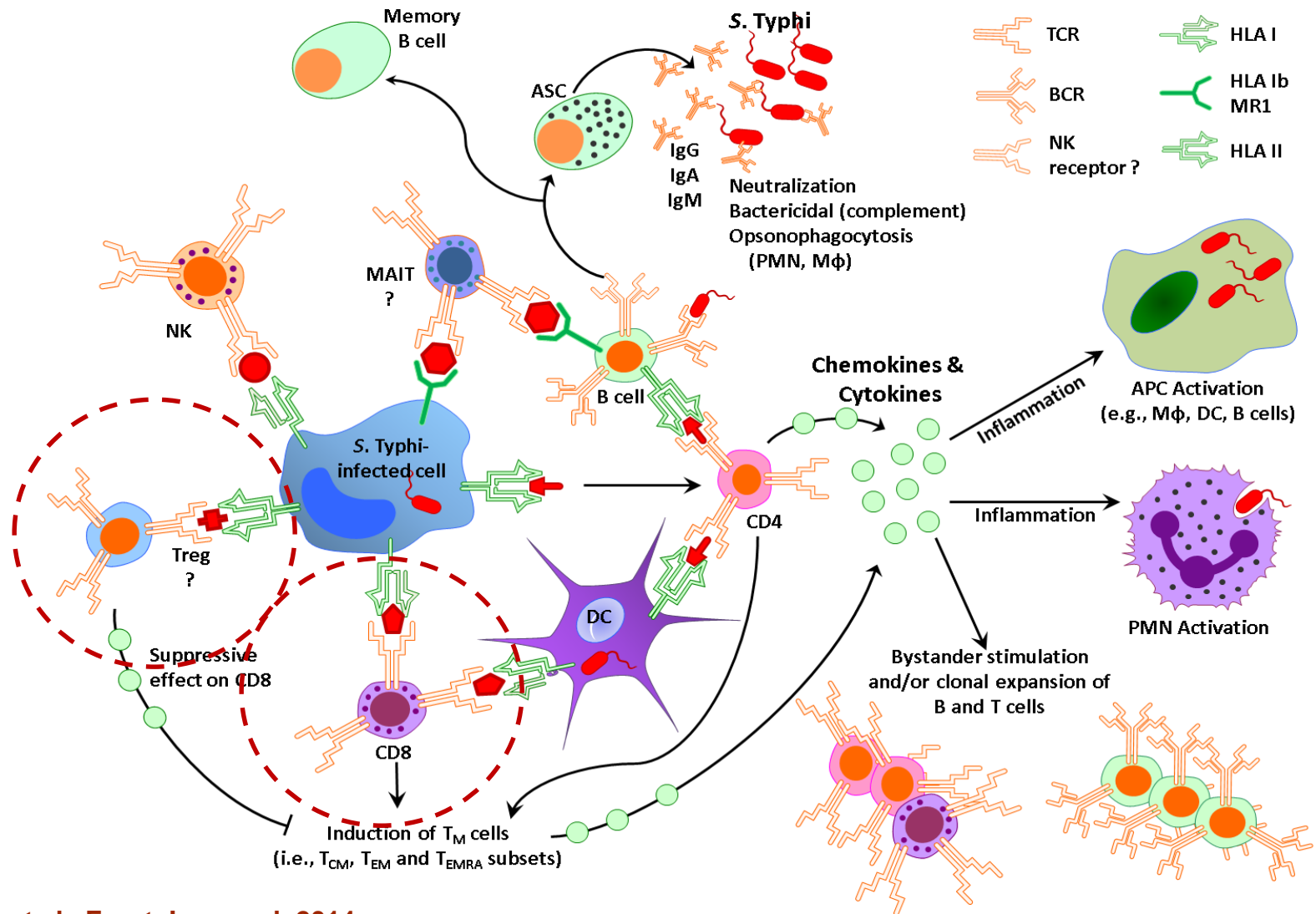
CMI to *S. Typhi*-infected autologous cells in Ty21 vaccinees: Multifunctional T cells

- **Why study multi-functionality of the T cell responses following Ty21a immunization?**
 - Multifunctional T cells (those producing ≥ 2 cytokines simultaneously, might be critical effectors in protection from infection in animals and humans (e.g., HIV, Mtb, Ebola) and be key determinants in long-term immunity
 - Technological advances and unsupervised flow cytometry analysis packages enable the study of all possible combinations of many cytokines to define multifunctional CD8⁺ T cell subsets

IL-17A production to *S. Typhi*-infected autologous cells in Ty21 vaccinees

- **Relevance:** IL-17A is a pro-inflammatory cytokine produced by CD4+ and CD8+ T cells shown to play a key role in mucosal immunity
- **Findings:**
 - ❖ First demonstration of IL-17A production by CD4+ & CD8+ (T_{EM} and T_{EMRA}) subsets elicited by Ty21a immunization
 - ❖ Responses were multifunctional and multiphasic (biphasic, triphasic over 1 year). Thus, evaluating a single time point may fail to accurately evaluate responses

Role of T_{eff} , T_{reg} , pT_{fh} , MAIT and T_{RM} systemically and in the gut mucosa in typhoid fever



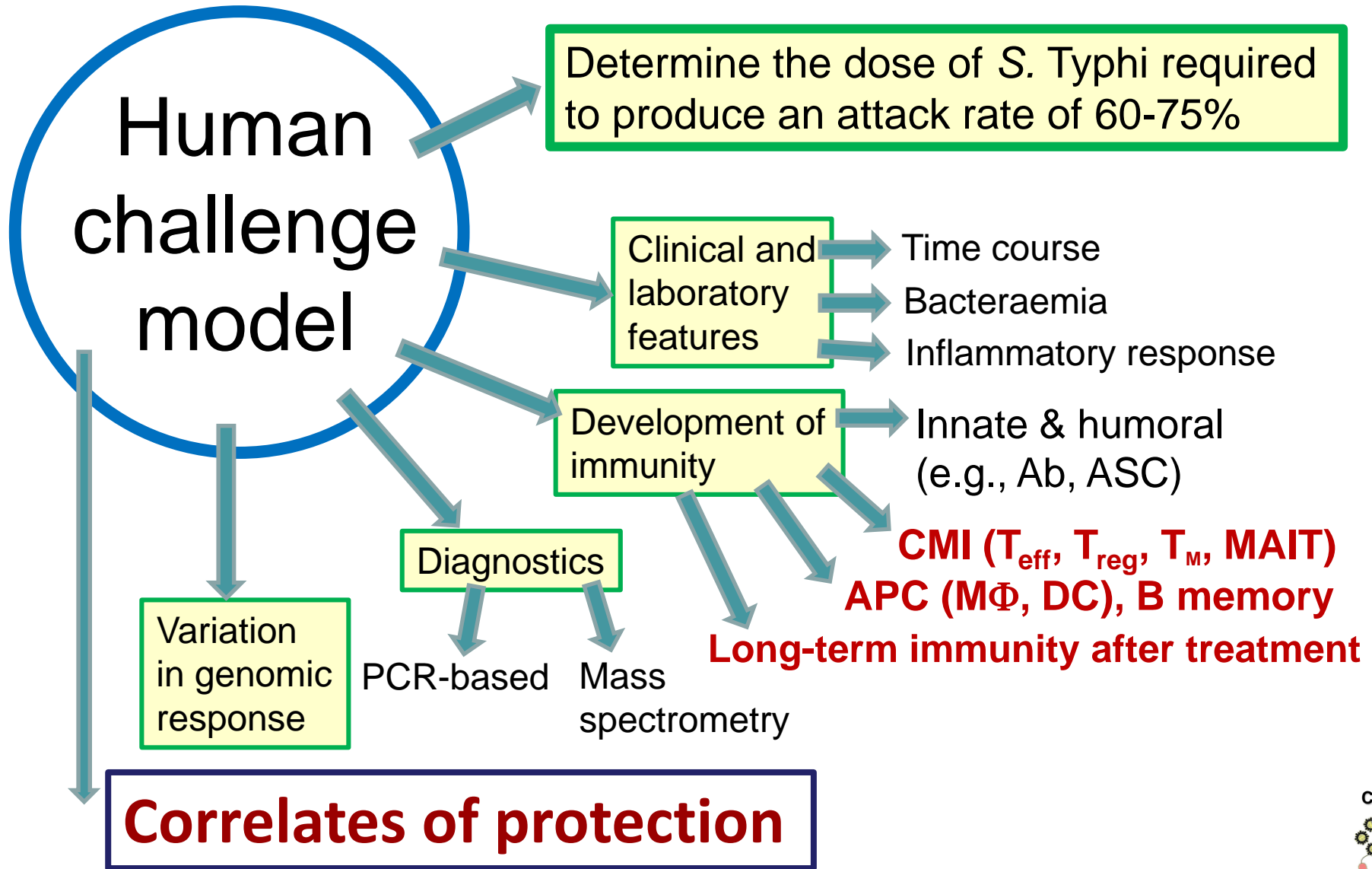
Immunity to *S. Typhi*: What is relevant?

Over the past 2 decades we demonstrated that immunization of volunteers with *S. Typhi* vaccines elicits complex and heterogeneous CMI responses

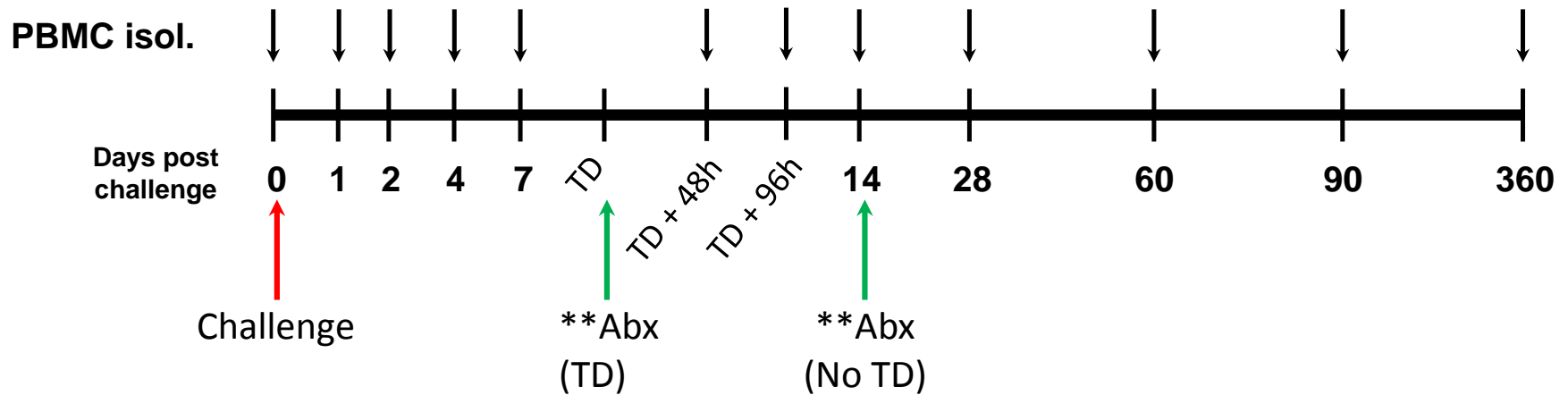
However, a key question remains unanswered: which of these CMI responses, if any, are associated with protection from typhoid fever?

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*

Goals of the wt *S. Typhi* human challenge model



Oxford challenge (study 1): Design

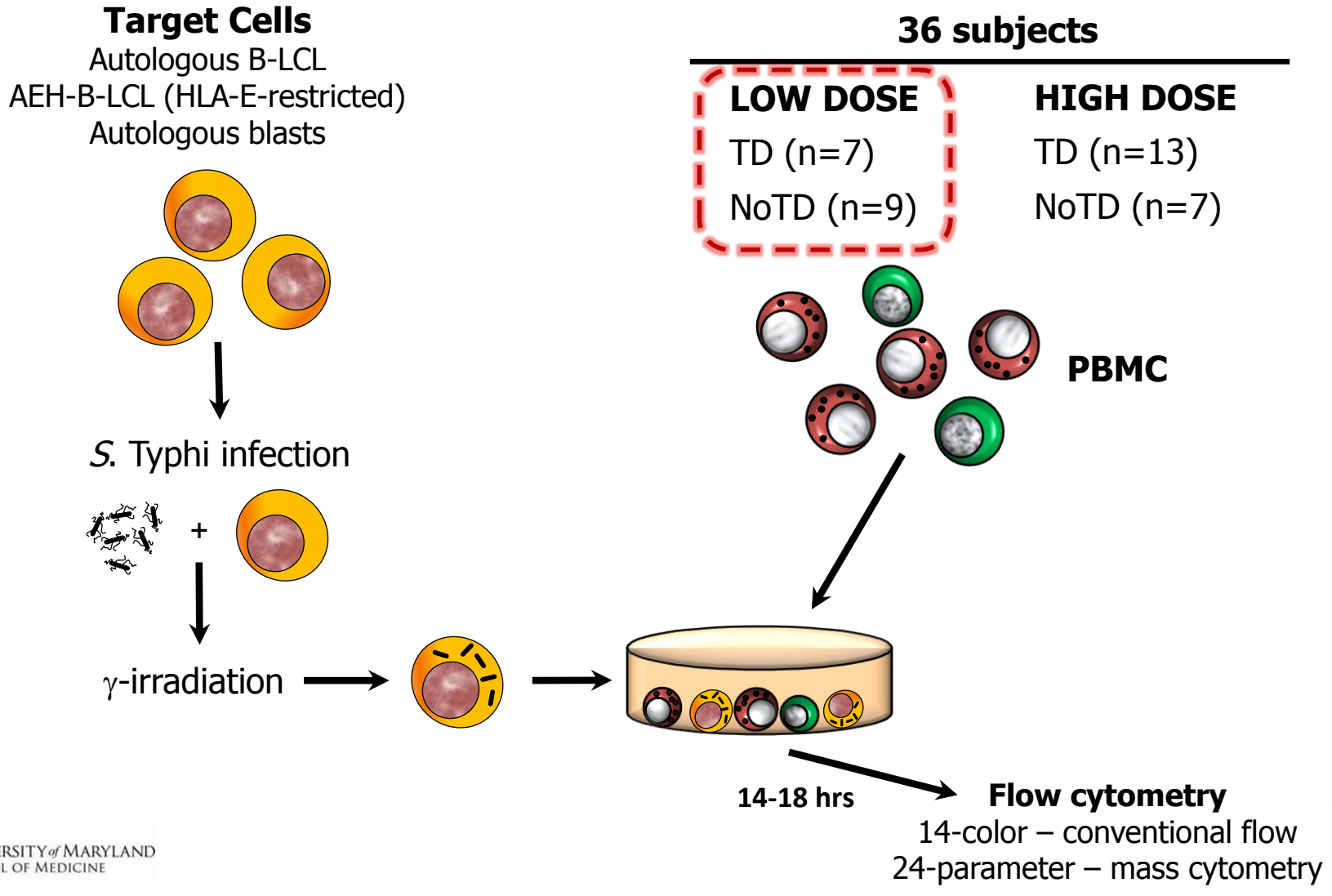


- 41 healthy participants received wt *S. Typhi*
- 2 doses evaluated (20 vol each) (**low** ($\sim 10^3$ cfu)) and **high** ($\sim 10^4$ cfu)
- Typhoid Diagnosis (TD) determined by fever $>38^\circ\text{C}$ for ≥ 12 hr or bacteremia
- Outcome: 61% (overall 25/41 developed TD)
- TD developed between 6-14 days after challenge
- Participants received antibiotics at time of diagnosis or at day 14

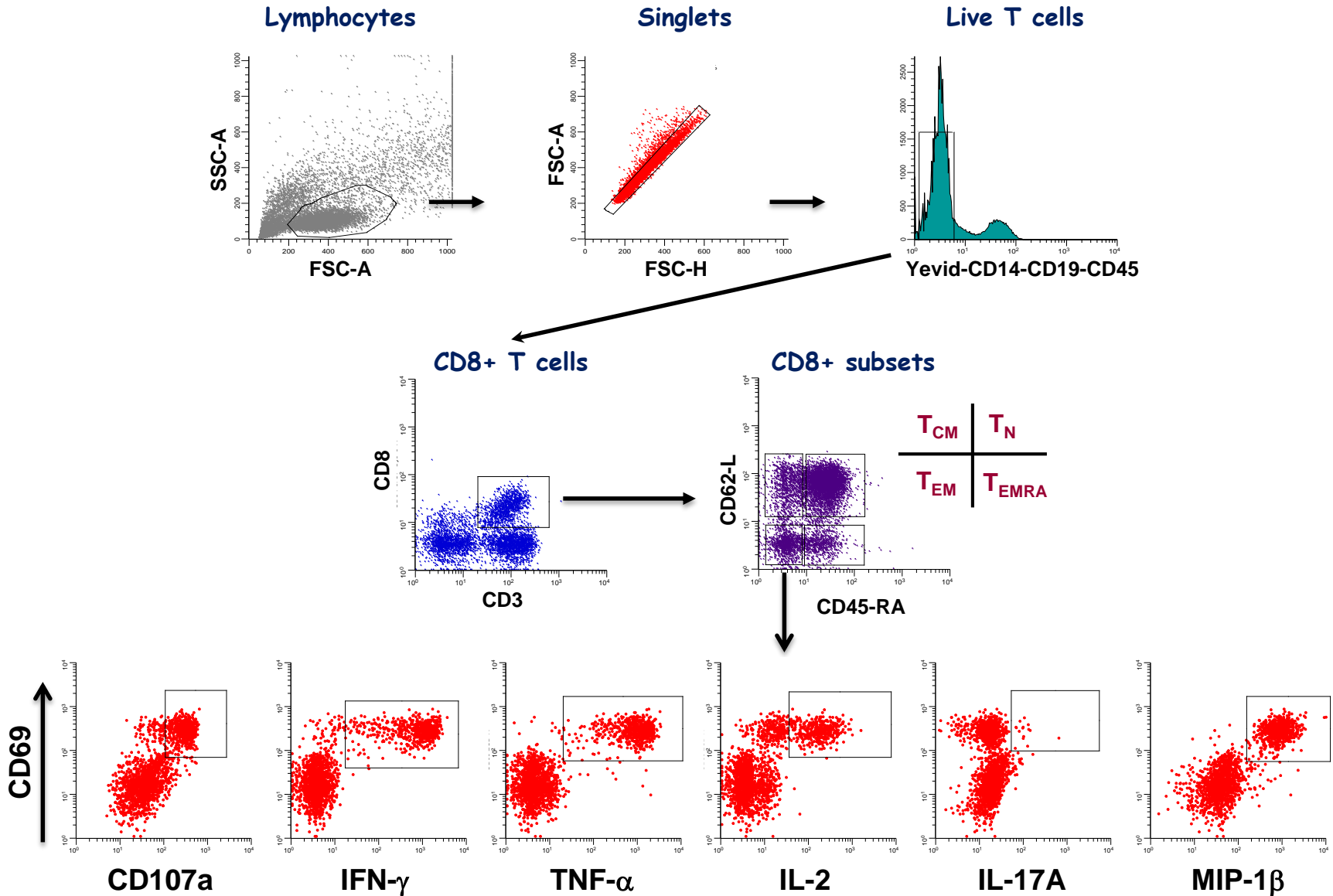
Oxford human wild-type *S.* Typhi challenge studies:

Memory & effector T cells

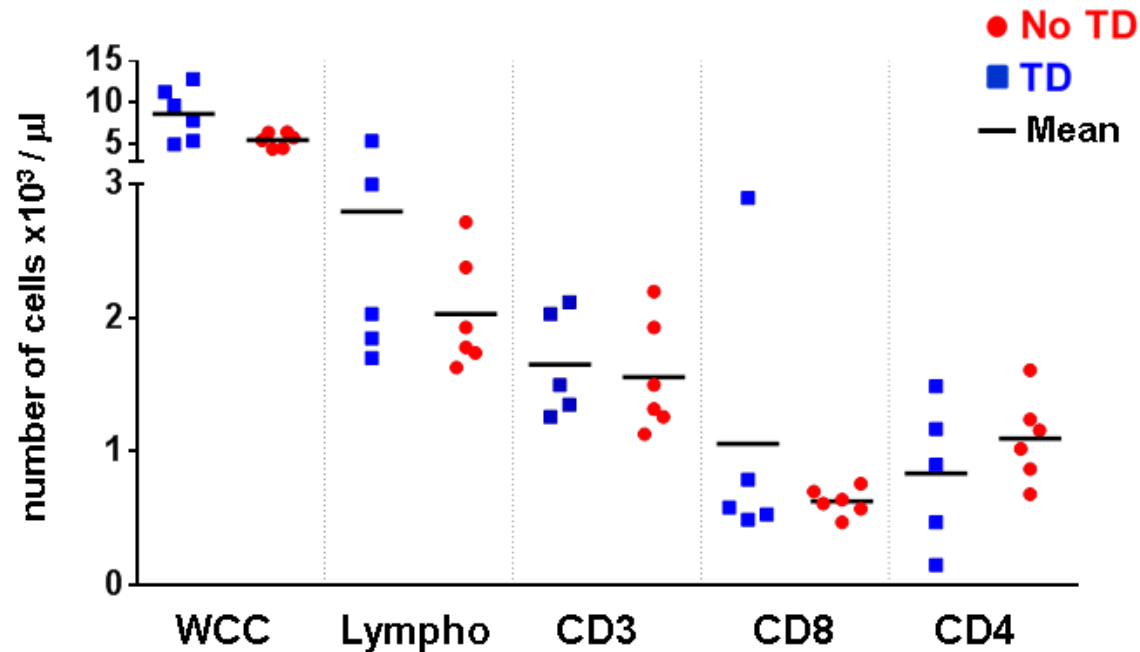
Experimental Design: in vitro stimulation of T effectors with *S. Typhi*-infected targets



Experimental Design: Flow cytometry gating



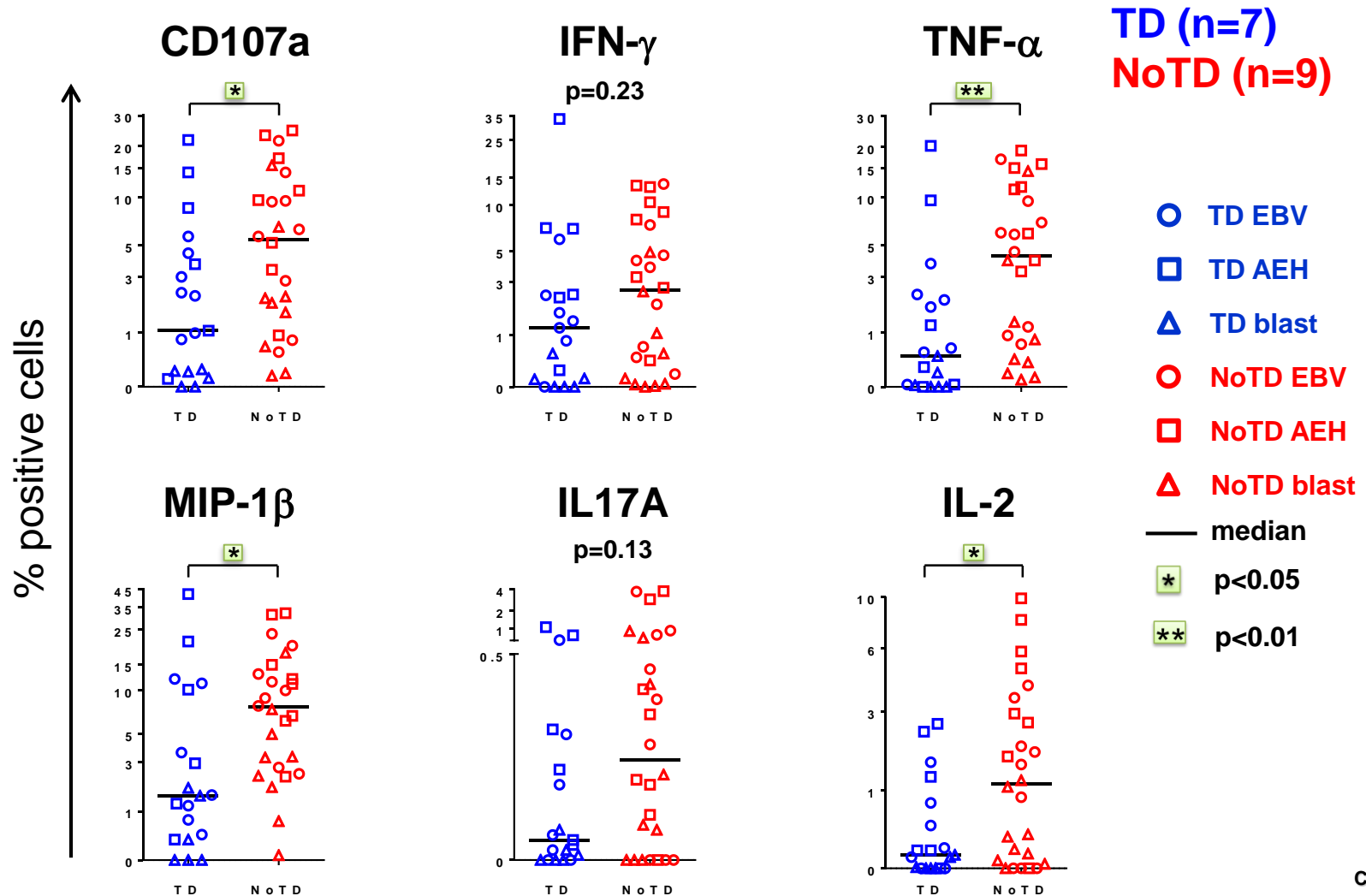
Absolute cell counts at baseline in TD and No TD



No differences were observed at baseline (pre-challenge) in the absolute numbers of WCC, lymphocytes, CD3, CD4 or CD8 cells between TD and No TD subjects

S. Typhi-specific CD8+ T_{EM} responses: Baseline

EBV, AEH & Blasts combined (low dose)



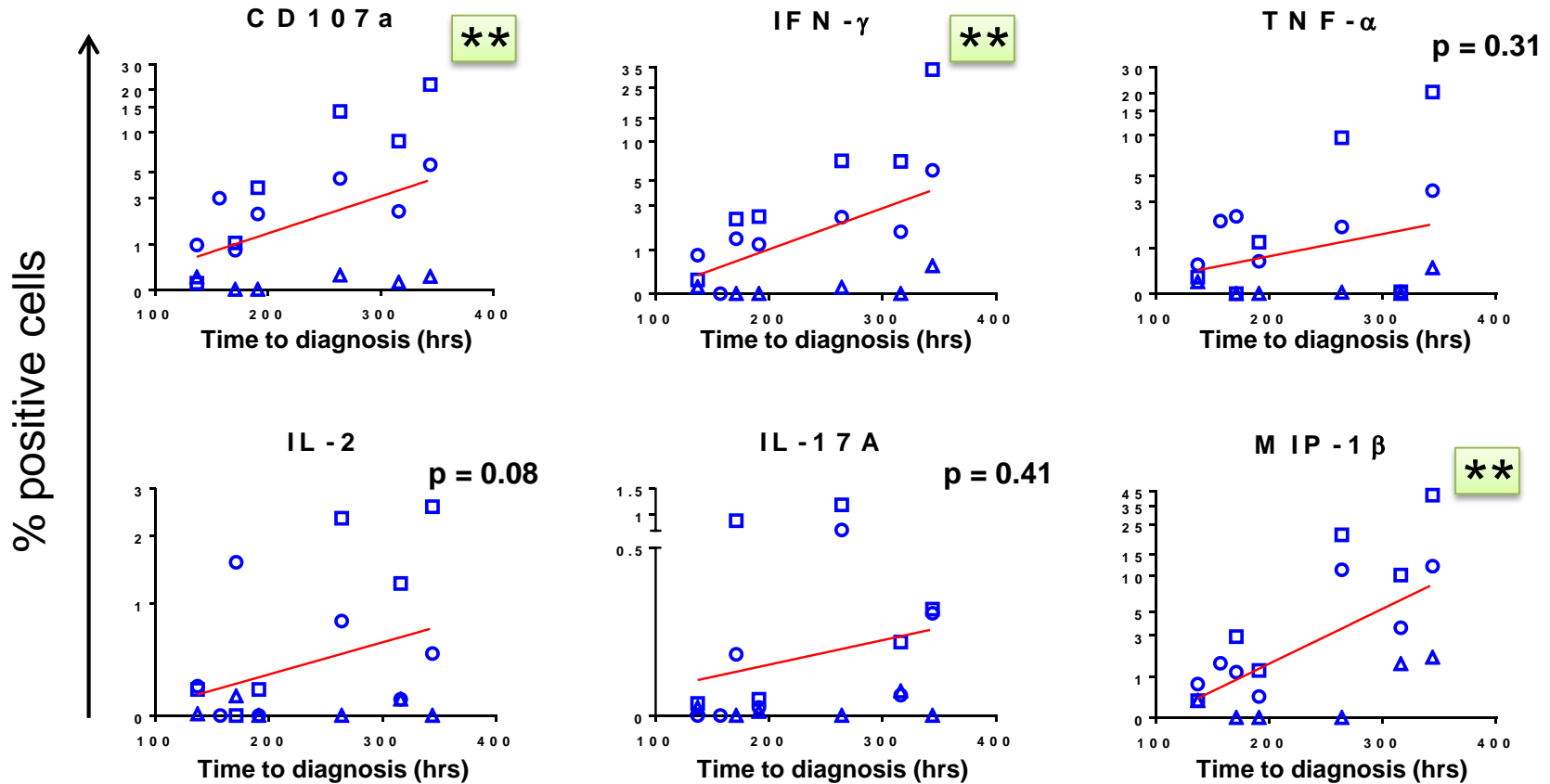
Higher baseline responses (pre-challenge) are associated with protection



Relationship between *S. Typhi*-specific baseline responses and time to diagnosis

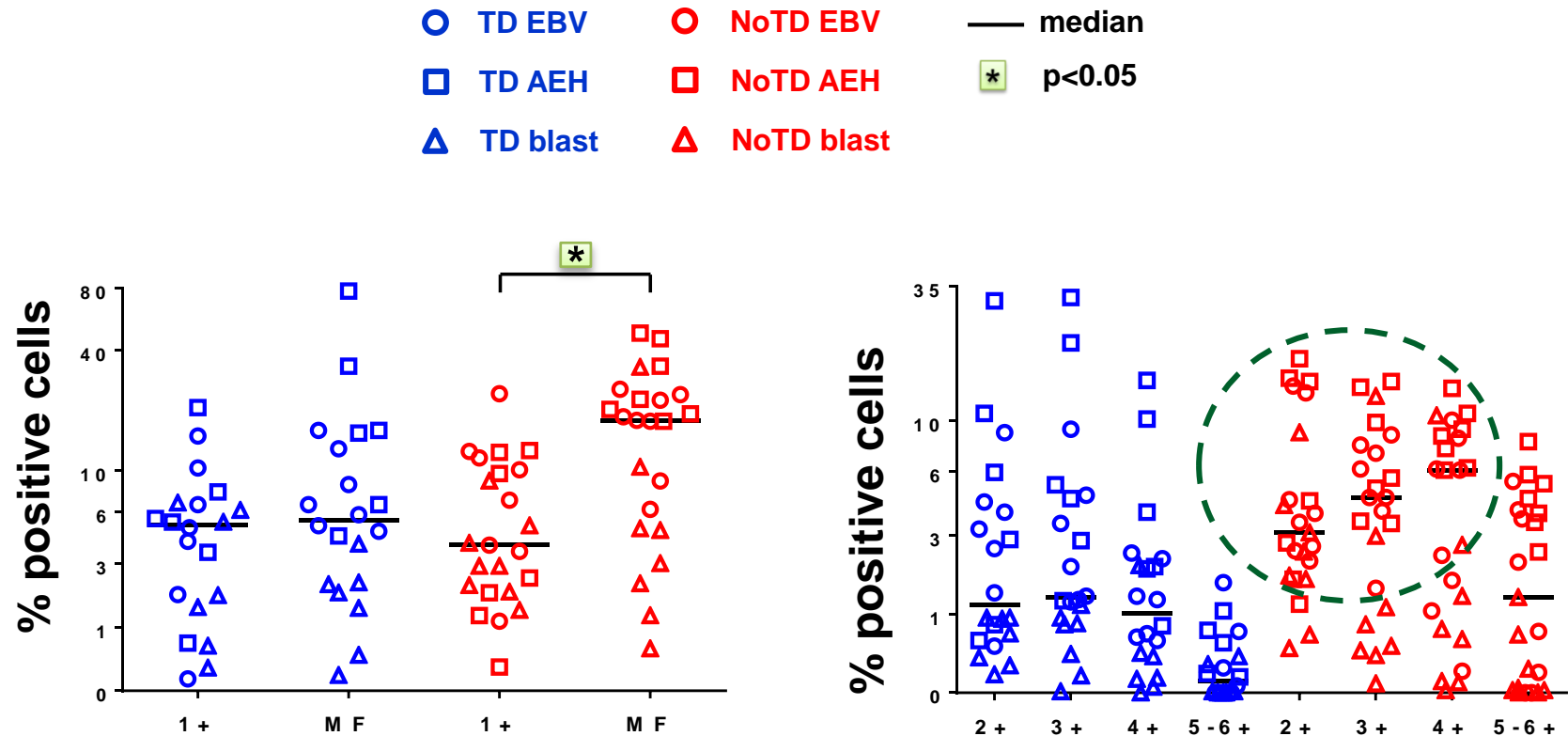
** p<0.01

EBV, AEH & Blasts combined (low dose)



High pre-challenge *S. Typhi*-specific baseline responses are associated with delayed time to diagnosis

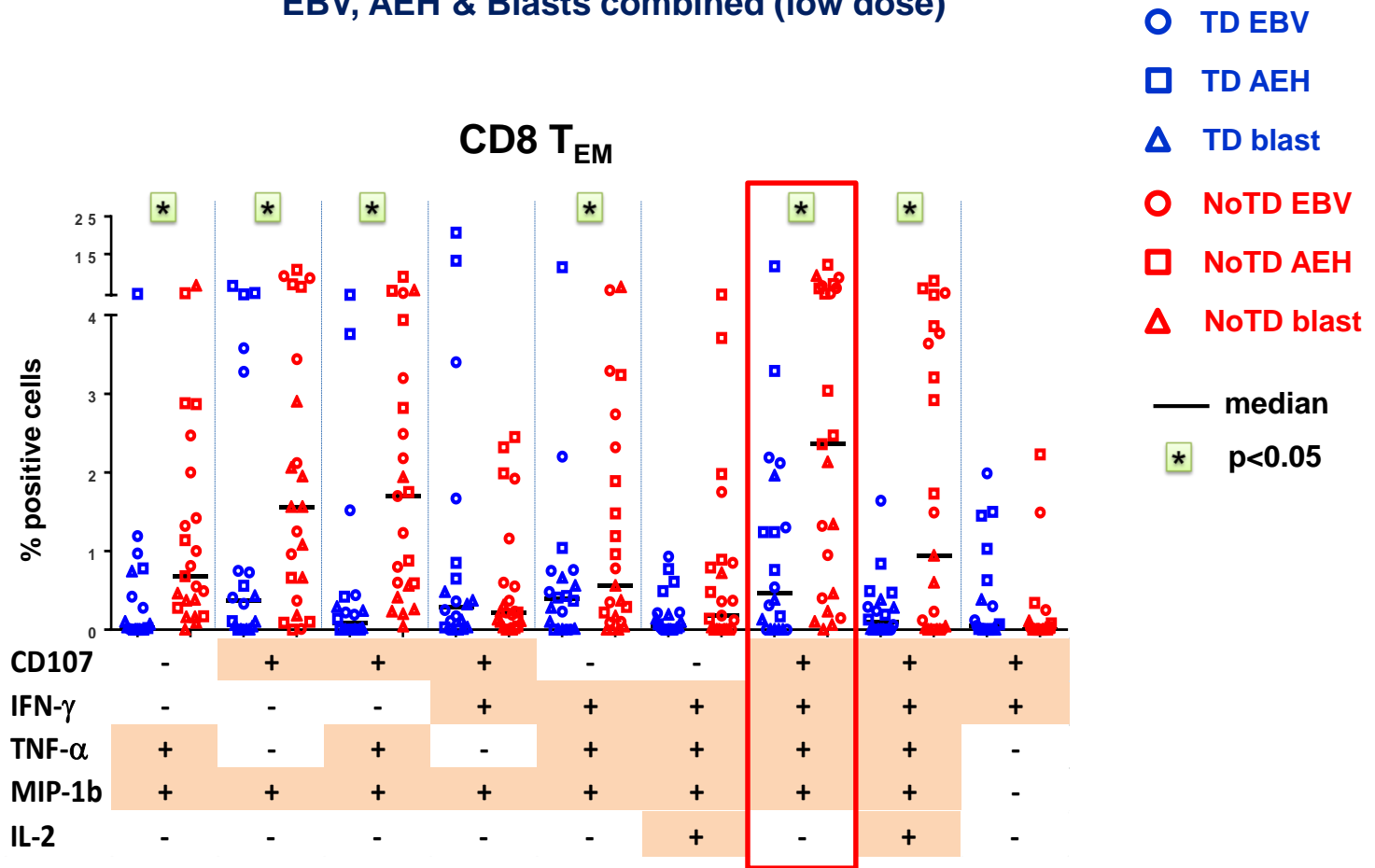
Multifunctional CD8+ T_{EM} responses: Baseline



MF S. Typhi-specific responses are dominant in NoTD volunteers

Multifunctional CD8+ T_{EM} responses: Baseline

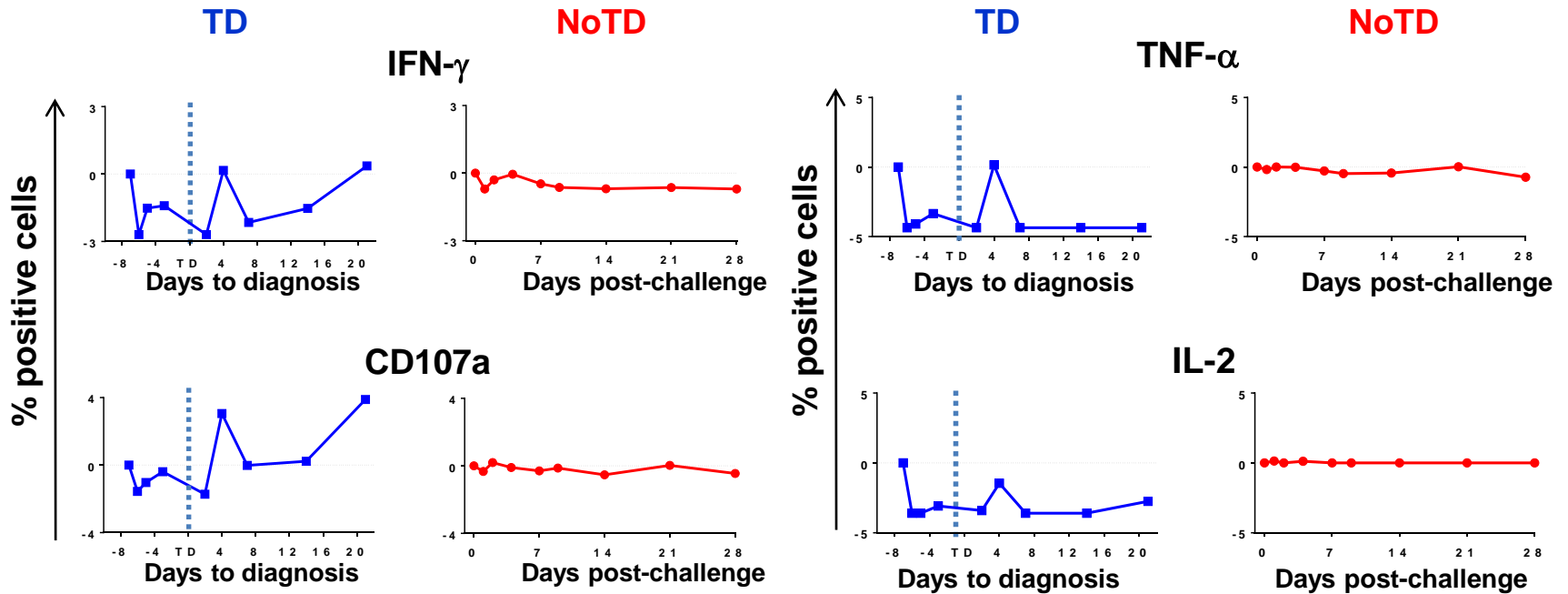
EBV, AEH & Blasts combined (low dose)



MF S. Typhi-specific responses are dominant in NoTD volunteers

Post-challenge kinetics of *S. Typhi*-specific CD8+ T_{EM} cell responses differs with clinical outcome

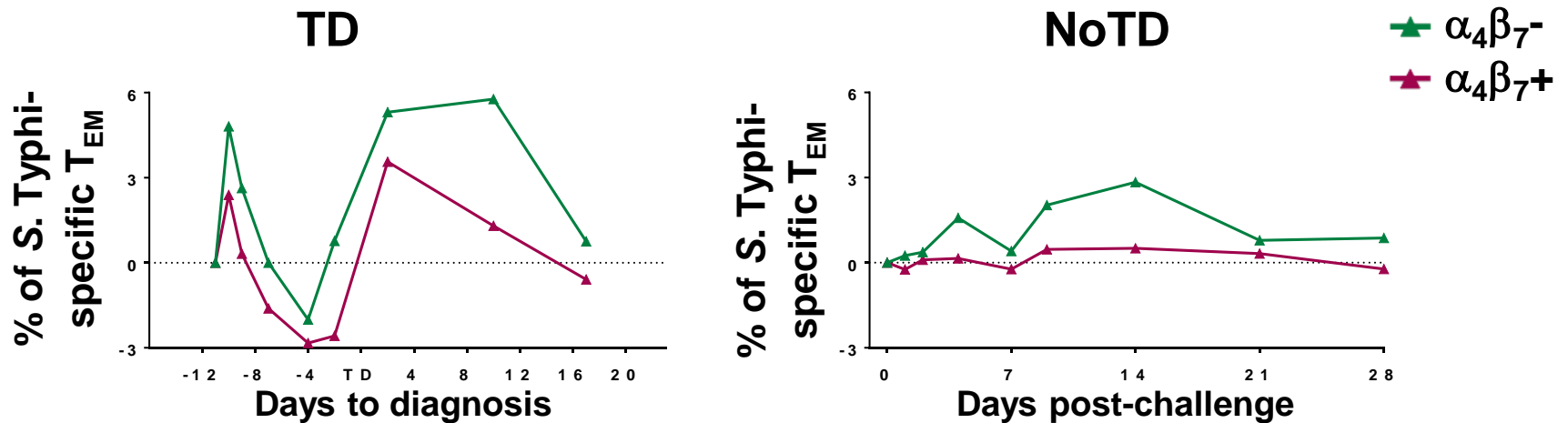
B-EBV



Marked decreases were observed in *S. Typhi*-specific T_{EM} expressing CD107a and producing cytokines following challenge and before the onset of disease

Post-challenge kinetics of *S. Typhi*-specific CD8+ T_{EM} expressing or not integrin $\alpha_4\beta_7$

B-EBV



Marked decreases were observed in *S. Typhi*-specific T_{EM} expressing, or not, integrin $\alpha_4\beta_7$ following challenge and before the onset of disease

T memory & effector cells: Conclusions

This study provides unique insights into the human immune response during the development of typhoid fever

- Uncovered, for the first time, that *S. Typhi*-specific CD8 T cell baseline responses correlate significantly with clinical outcome after infection. Higher baseline *S. Typhi*-specific responses are associated with:
 - Protection from typhoid disease
 - Delayed time to diagnosis in subjects who developed TD
- Demonstrated that multifunctional T cells are likely to **play an important** role in protection from the development of typhoid disease

RESEARCH

Open Access



Salmonella Typhi-specific multifunctional CD8⁺ T cells play a dominant role in protection from typhoid fever in humans

Stephanie Fresnay¹, Monica A. McArthur¹, Laurence Magder², Thomas C. Darton³, Claire Jones³,
Claire S. Waddington³, Christoph J. Blohmke³, Brian Angus⁴, Myron M. Levine¹, Andrew J. Pollard³
and Marcelo B. Sztein^{1*}

Oxford human wild-type *S.* Typhi challenge studies:

Regulatory T cells

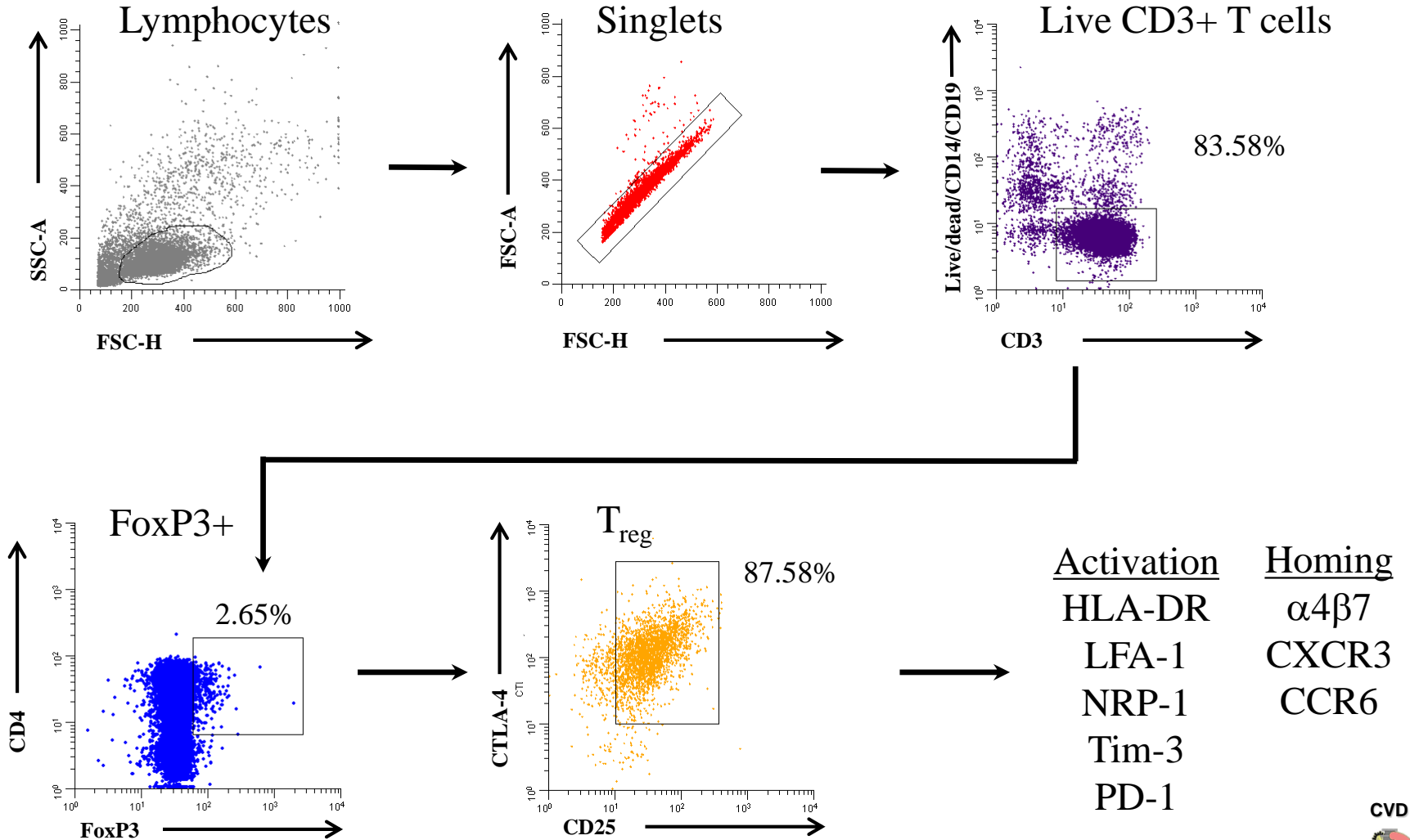
Regulatory T cells (T_{reg})

- Subset of CD4+ T cells that suppress other immune cells
- Characterized by expression of IL-2R (CD25) and transcription factor FoxP3
- Traffic to sites of specific immune responses
- Regulatory functions
 - CTLA-4 competition for co-stimulatory molecules (CD80 and CD86) on antigen presenting cells (APC)
 - Consumption of IL-2 (IL-2R)
 - Cytokine production (IL-10)

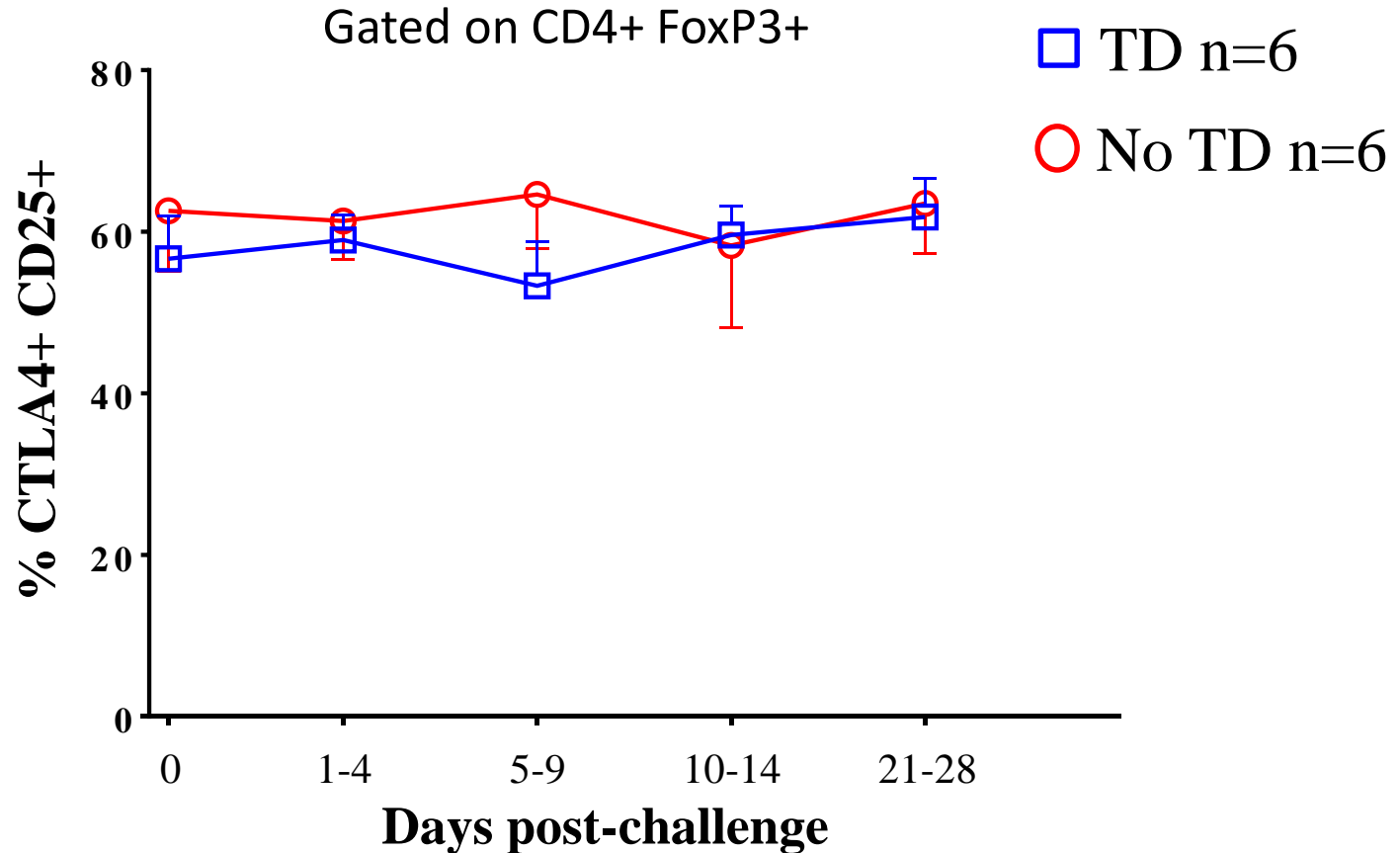
Goals

- Identify the potential role of circulating T_{reg} in a wild-type *S. Typhi* challenge model in humans
- Identify the homing potential and activation characteristics of T_{reg} associated with typhoid diagnosis

Gating Strategy

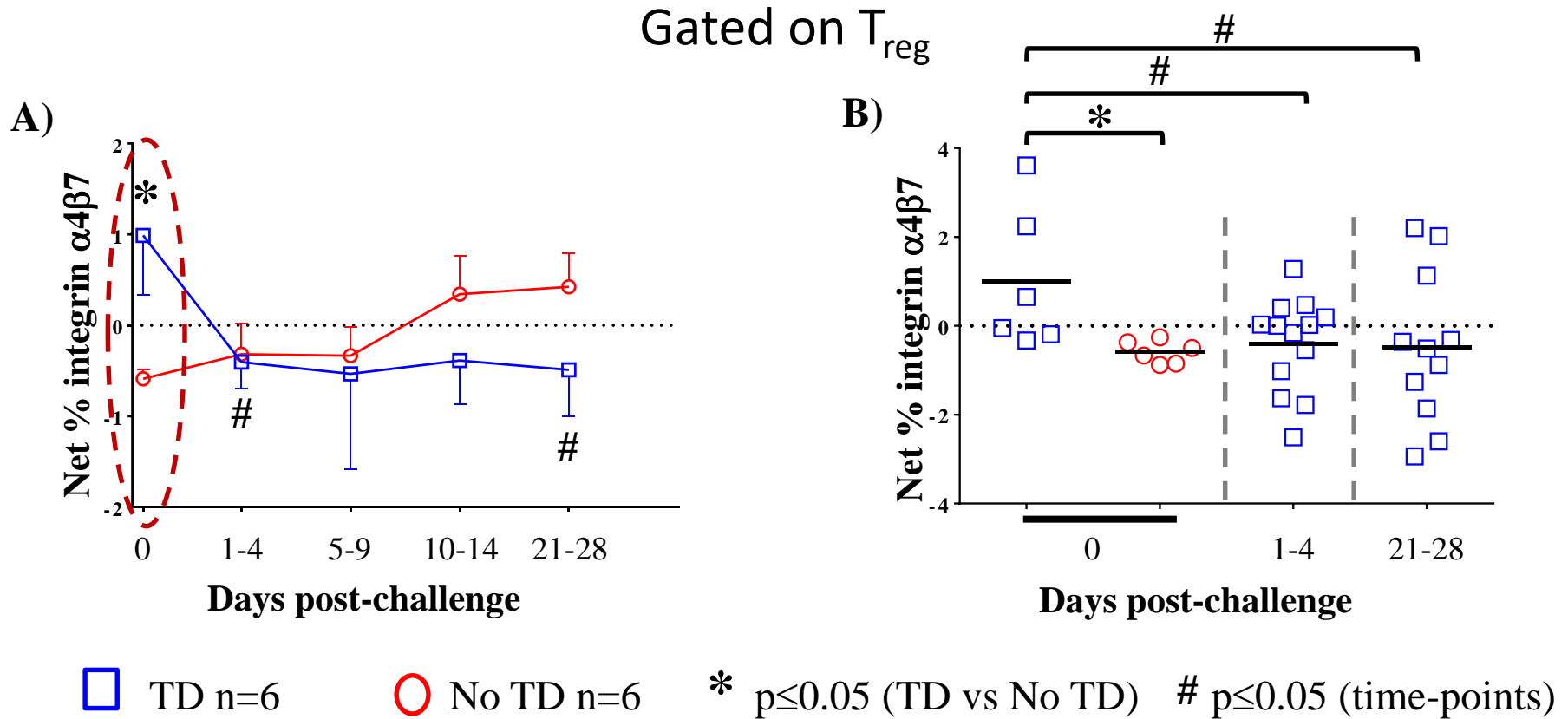


Total circulating T_{reg}



No significant difference in mean percentages of total T_{reg} across time segments or between TD and No TD volunteers indicating that changes in total circulating Treg are not associated with TD

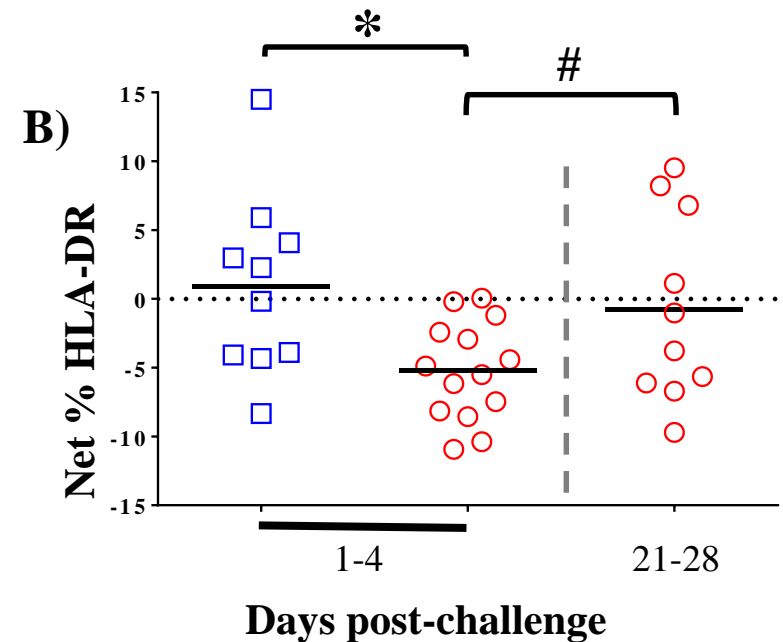
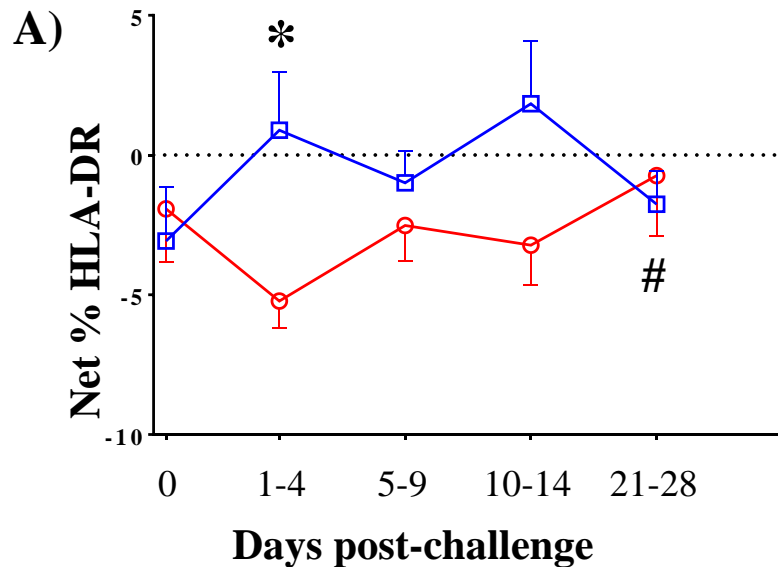
Gut homing potential of circulating T_{reg}



- **S. Typhi-specific expression of integrin $\alpha 4\beta 7$ is up-regulated on circulating Treg pre-challenge in TD volunteers indicating possible association with disease.**
- **Down-regulation of S. Typhi-specific integrin $\alpha 4\beta 7$ expression on Treg post-challenge in TD volunteers indicates potential homing to the gut**

S. Typhi-specific activation of circulating T_{reg}

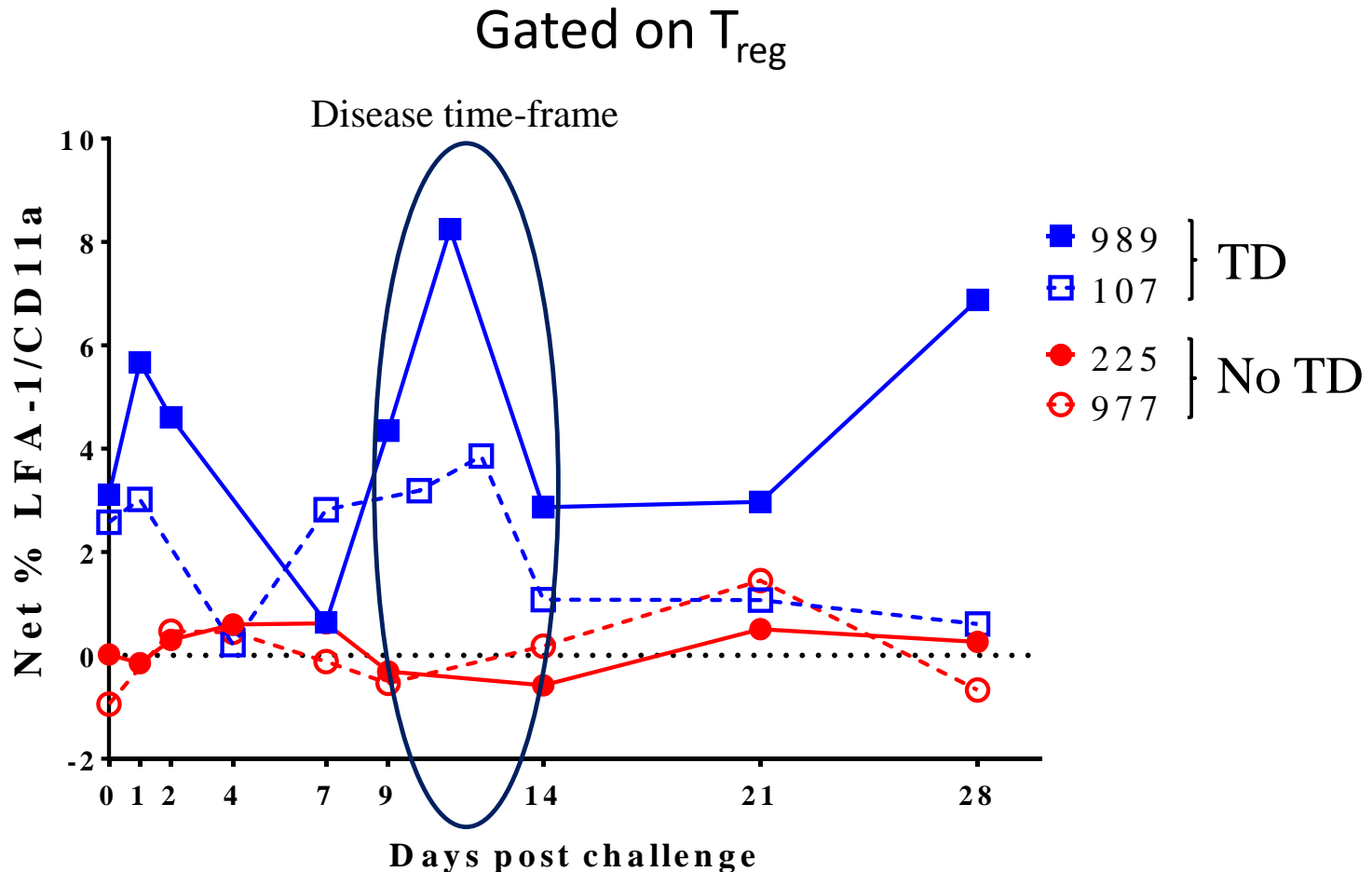
Gated on T_{reg}



□ TD n=6 ○ No TD n=6 * p≤0.05 (TD vs No TD) # p≤0.05 (time-points)

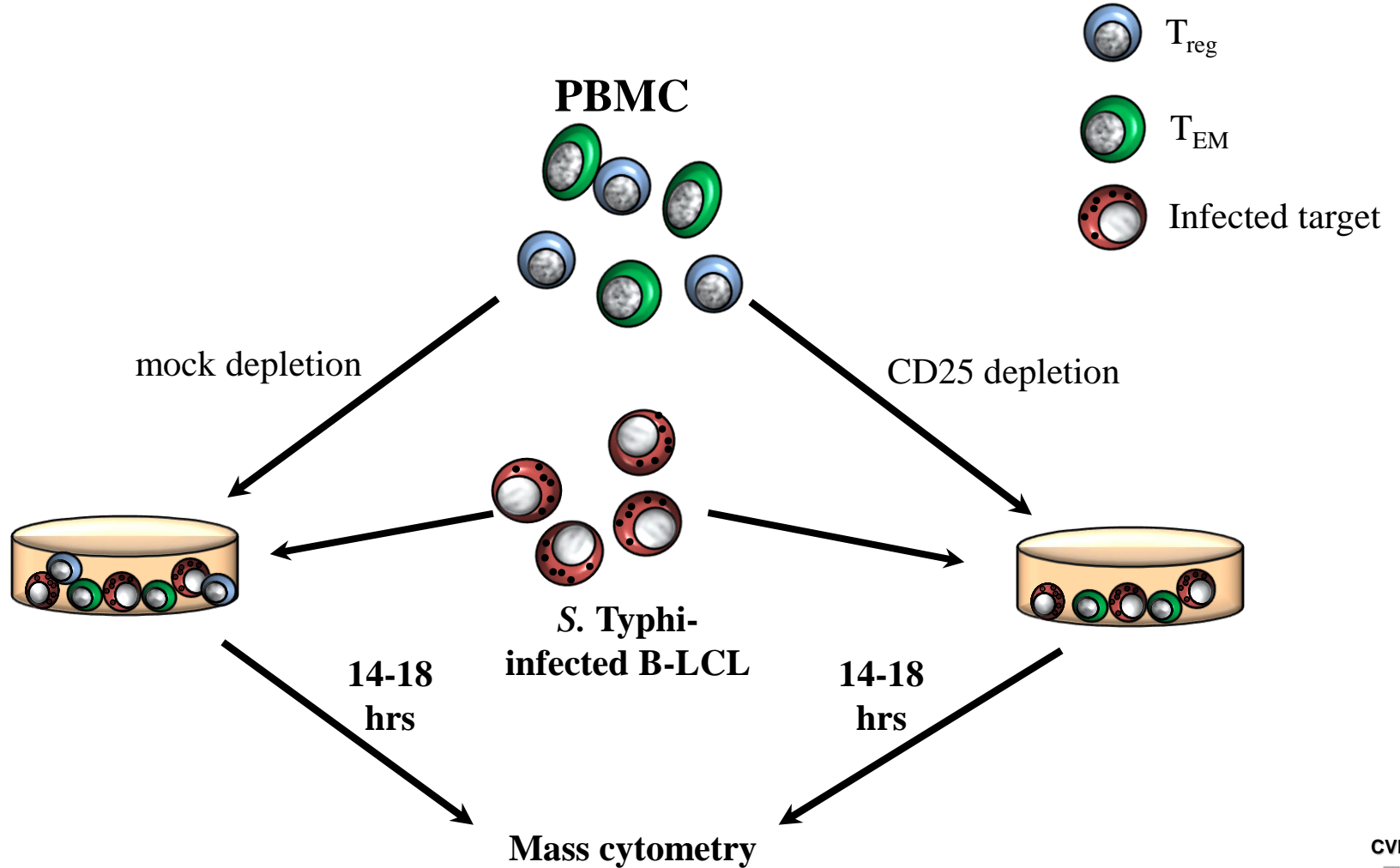
Higher S. Typhi-specific expression of activation molecules (HLA-DR) on T_{reg} post-challenge is associated with TD indicating S. Typhi-specific activation of T_{reg} may be associated with development of disease

Kinetics of *S. Typhi*-specific T_{reg} activation (representative volunteers)



TD volunteers have up-regulation of *S. Typhi*-specific expression of LFA-1 near the time of typhoid diagnosis supporting the association of increased activation of T_{reg} with TD

Depletion of T_{reg}

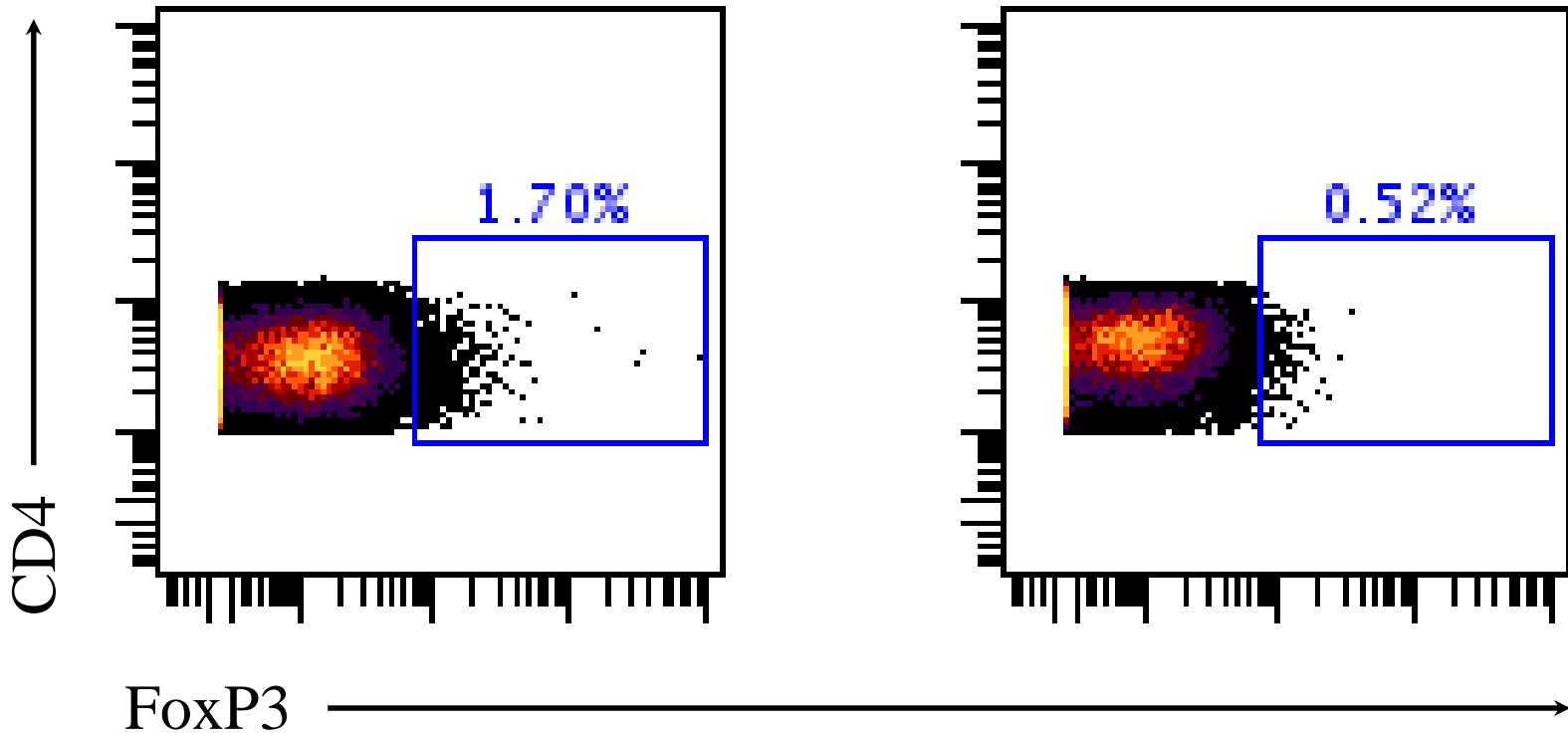


Depletion of T_{reg}

Gated on CD3+ CD4+

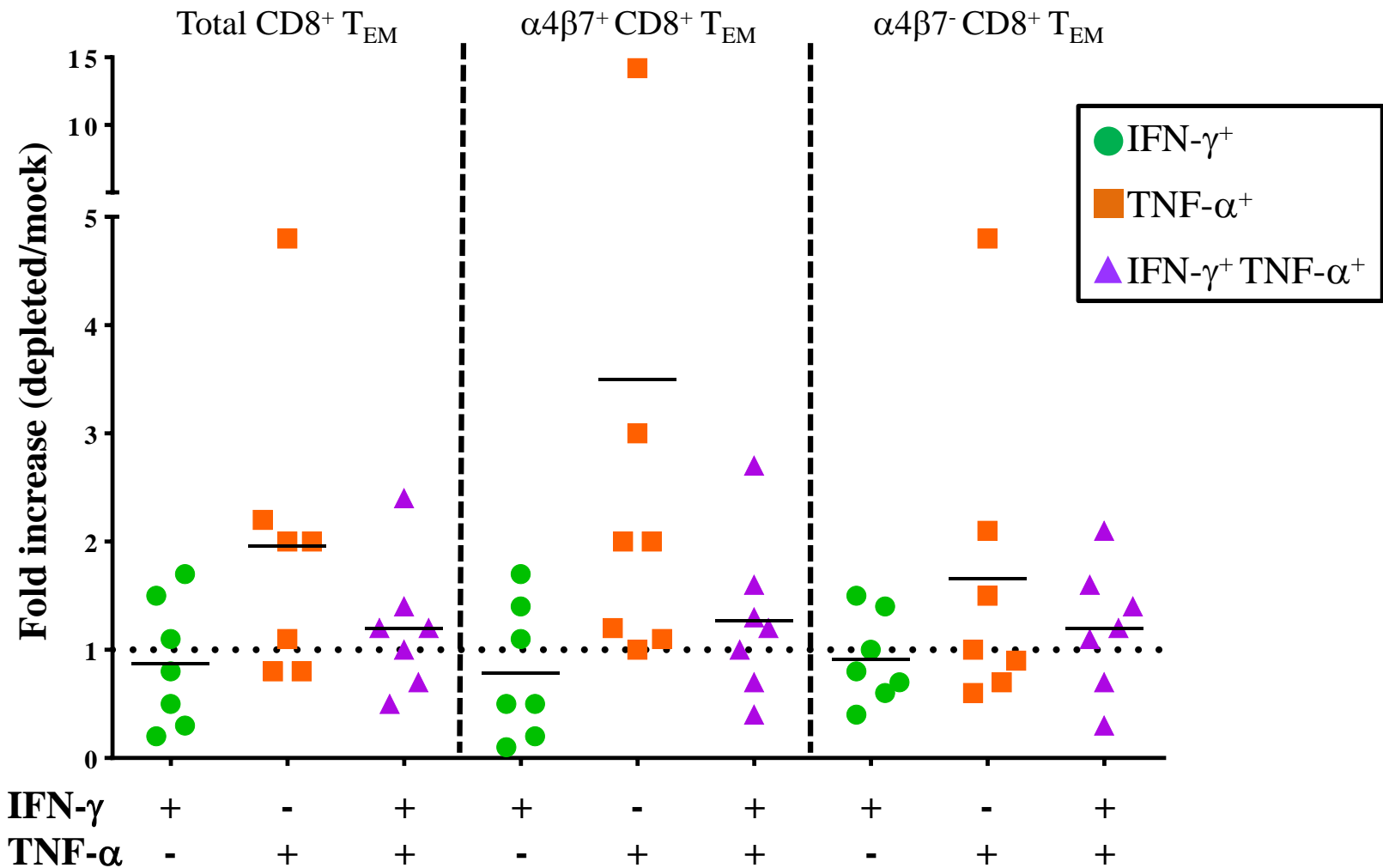
Mock depletion

CD25 depletion



CD25 depletion results in decreased levels of FoxP3+ Treg

S. Typhi-specific cytokine production after T_{reg} depletion



Cytokine production by *S. Typhi*-specific CD8⁺ T_{EM} is higher in the absence of T_{reg} indicating that T_{reg} may functionally suppress *S. Typhi*-specific responses

T regulatory cells: Conclusions (I)

- *S. Typhi*-specific expression of integrin $\alpha 4\beta 7$ is up-regulated pre-challenge in TD volunteers
- Down-regulation of *S. Typhi*-specific expression of integrin $\alpha 4\beta 7$ on circulating T_{reg} occurs post-challenge in TD volunteers
 - Higher levels of *S. Typhi*-specific gut homing potential may result in accumulation of T_{reg} in the local gut environment resulting in suppression of protective pro-inflammatory responses and TD

T regulatory cells: Conclusions (II)

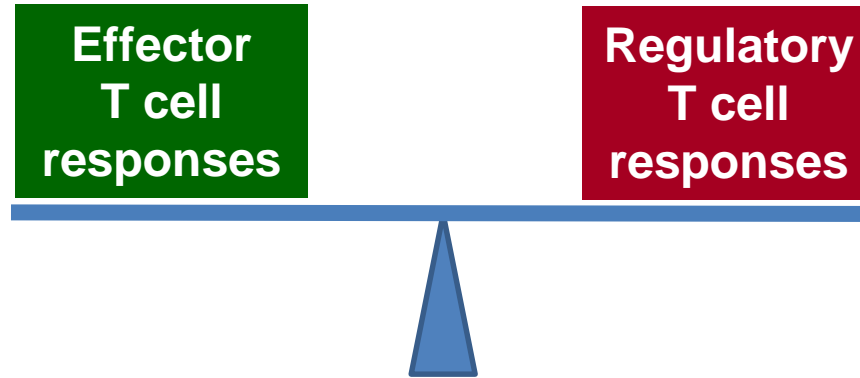
- *S.* Typhi-specific expression of activation molecules is increased in TD volunteers
- T_{reg} are capable of functionally suppressing *S.* Typhi-specific responses
- T_{reg} suppress lymphocytes with gut homing potential as well as those that are likely to remain in the periphery

RESEARCH ARTICLE

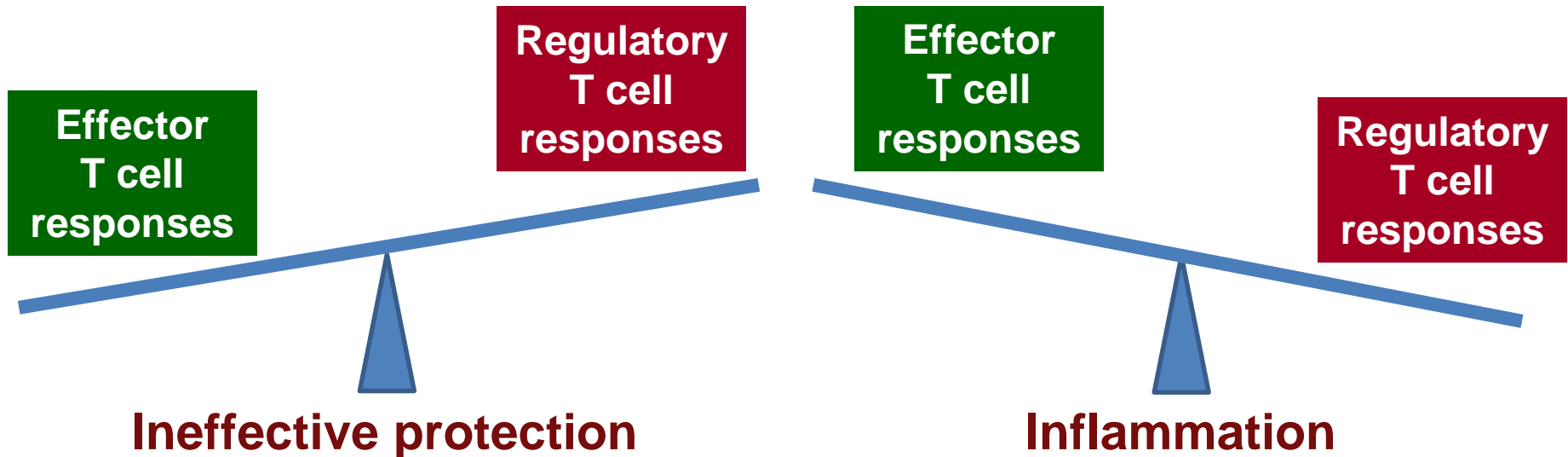
Activation of *Salmonella* Typhi-Specific Regulatory T Cells in Typhoid Disease in a Wild-Type *S. Typhi* Challenge Model

Monica A. McArthur¹, Stephanie Fresnay¹, Laurence S. Magder², Thomas C. Darton³, Claire Jones³, Claire S. Waddington³, Christoph J. Blohmke³, Gordon Dougan⁴, Brian Angus⁵, Myron M. Levine¹, Andrew J. Pollard³, Marcelo B. Sztein^{1*}

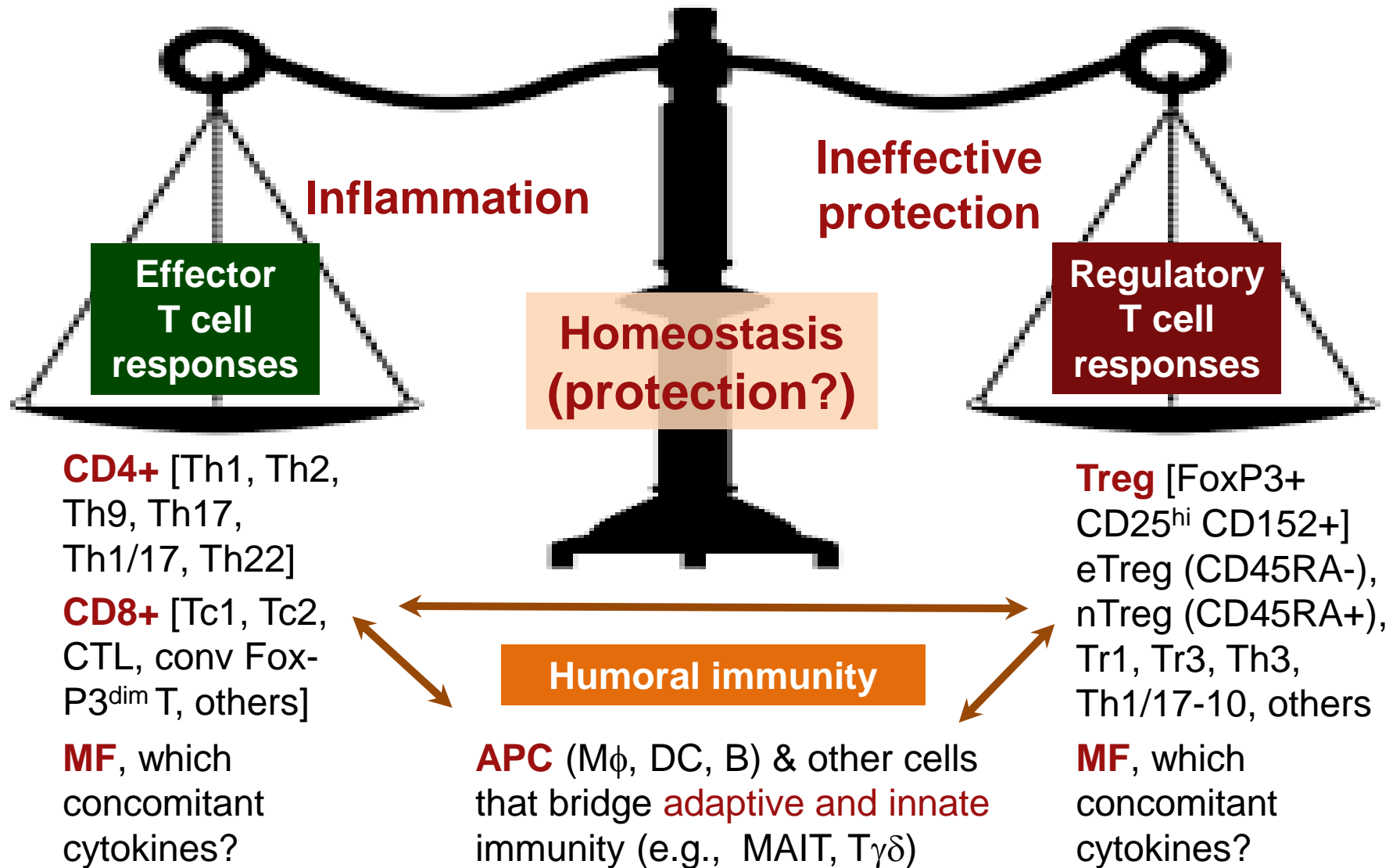
Balancing the immune response



Homeostasis (protection?)



Balancing the immune response



Host HLA and other genetic factors, nutrition, microbiota, etc

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

Thus, identifying the effective immunological CoP and their kinetics and homing among a multitude of non-protective or downregulatory immune responses might hold the key for the development of more effective attenuated enteric fever vaccines

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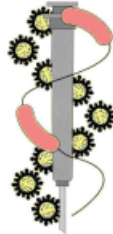
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