Recent advances in the identification of immunological correlates of protection in a human *S.* Typhi challenge model

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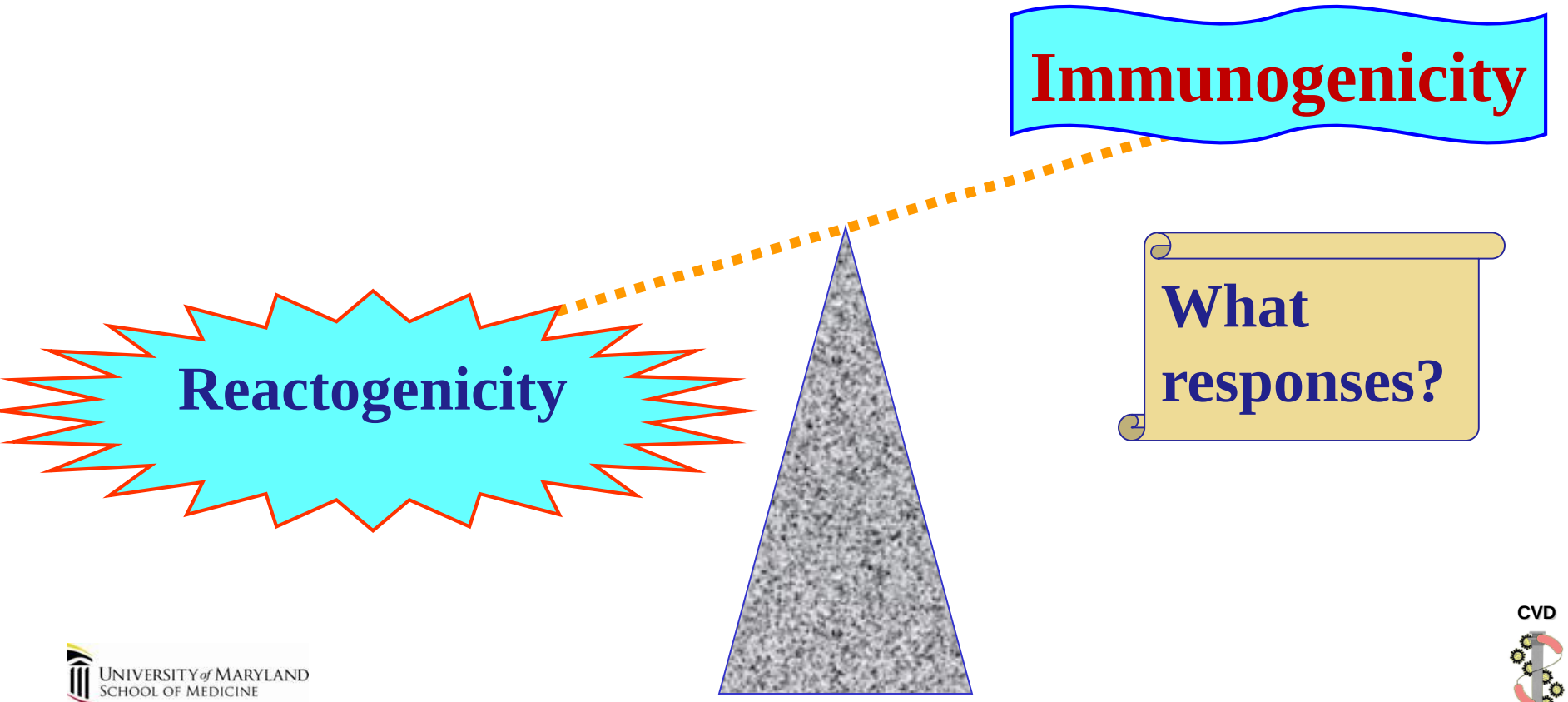
Vaccine Development

The never ending search for the optimal balance



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

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**

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

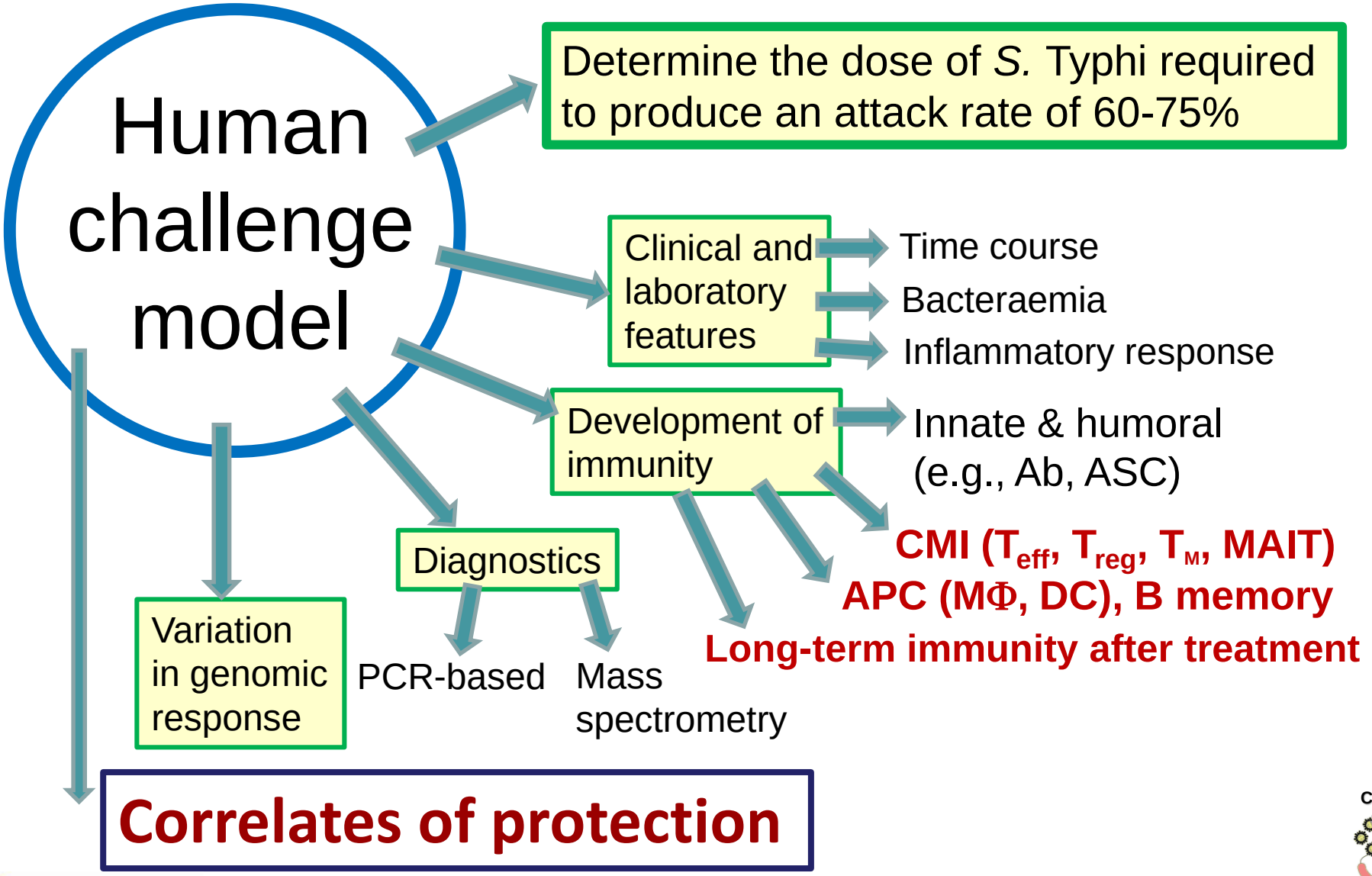
However, a key question remains unanswered: which of these CMI responses, if any, are associated with protection from typhoid fever?

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) and other enteric infections (e.g., *Shigella*, ETEC)

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

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



Oxford human wild-type *S.* Typhi challenge studies (Quailes strain)

Memory & effector T cells

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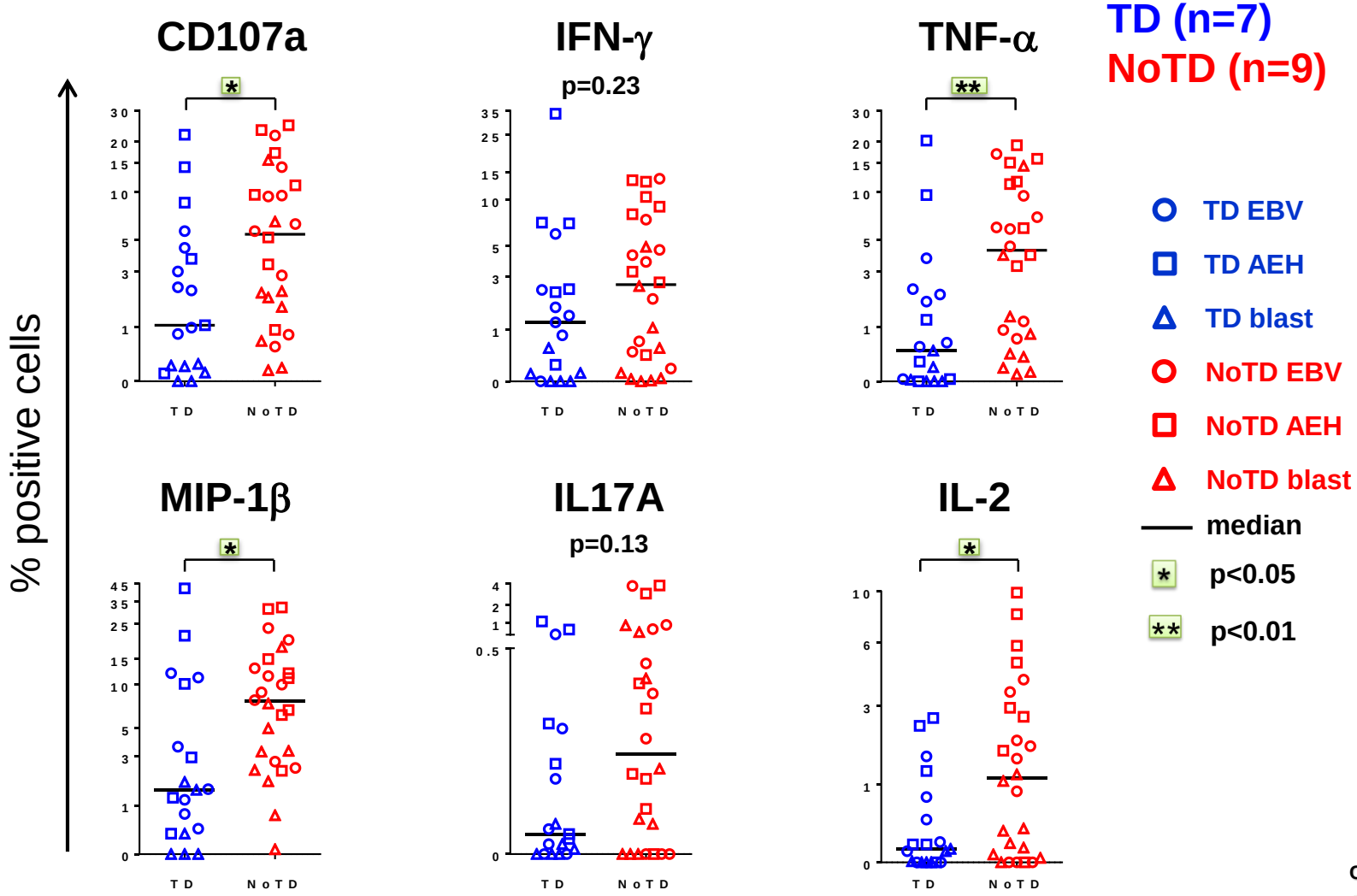
S. Typhi-responsive CD8+ T_{EM} responses: Baseline

EBV, AEH & Blasts combined (low dose)



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

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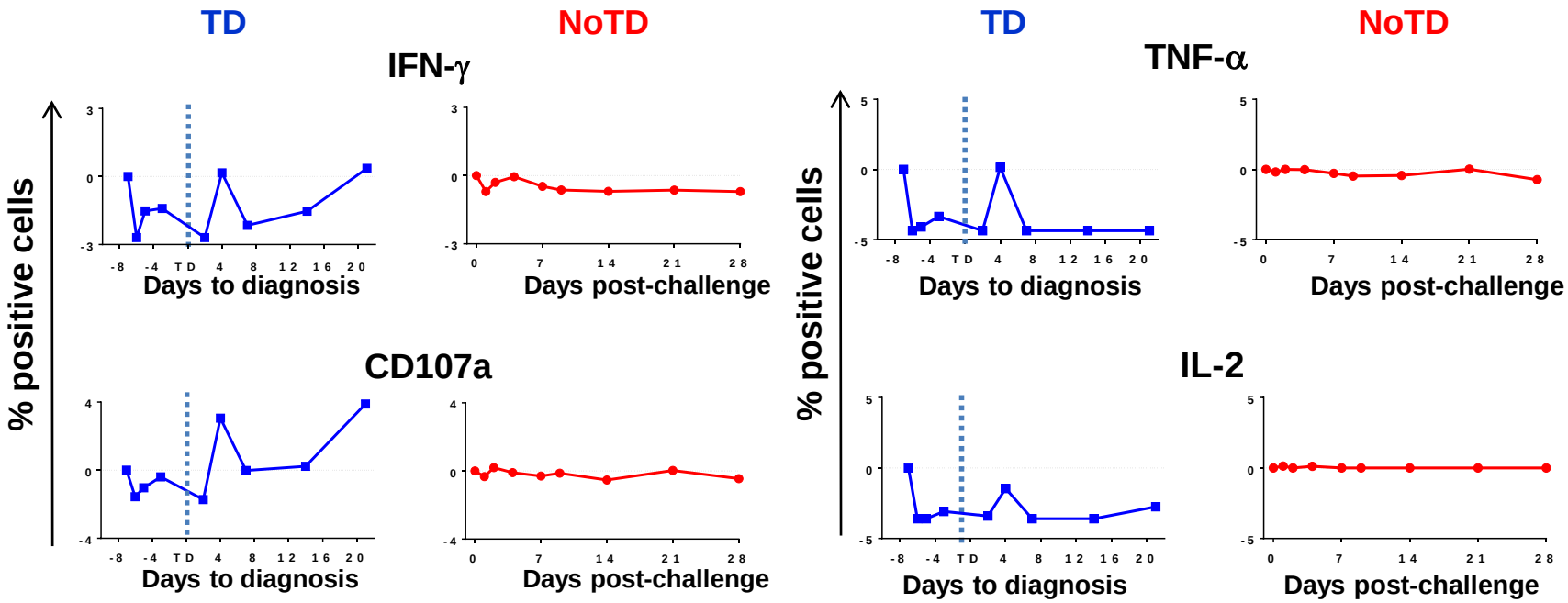
Post-challenge kinetics of *S.* Typhi-specific CD8+ T_{EM} cell responses differs with clinical outcome

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

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

Oxford human wild-type *S.* Typhi challenge studies (Quailes strain)

Regulatory T cells

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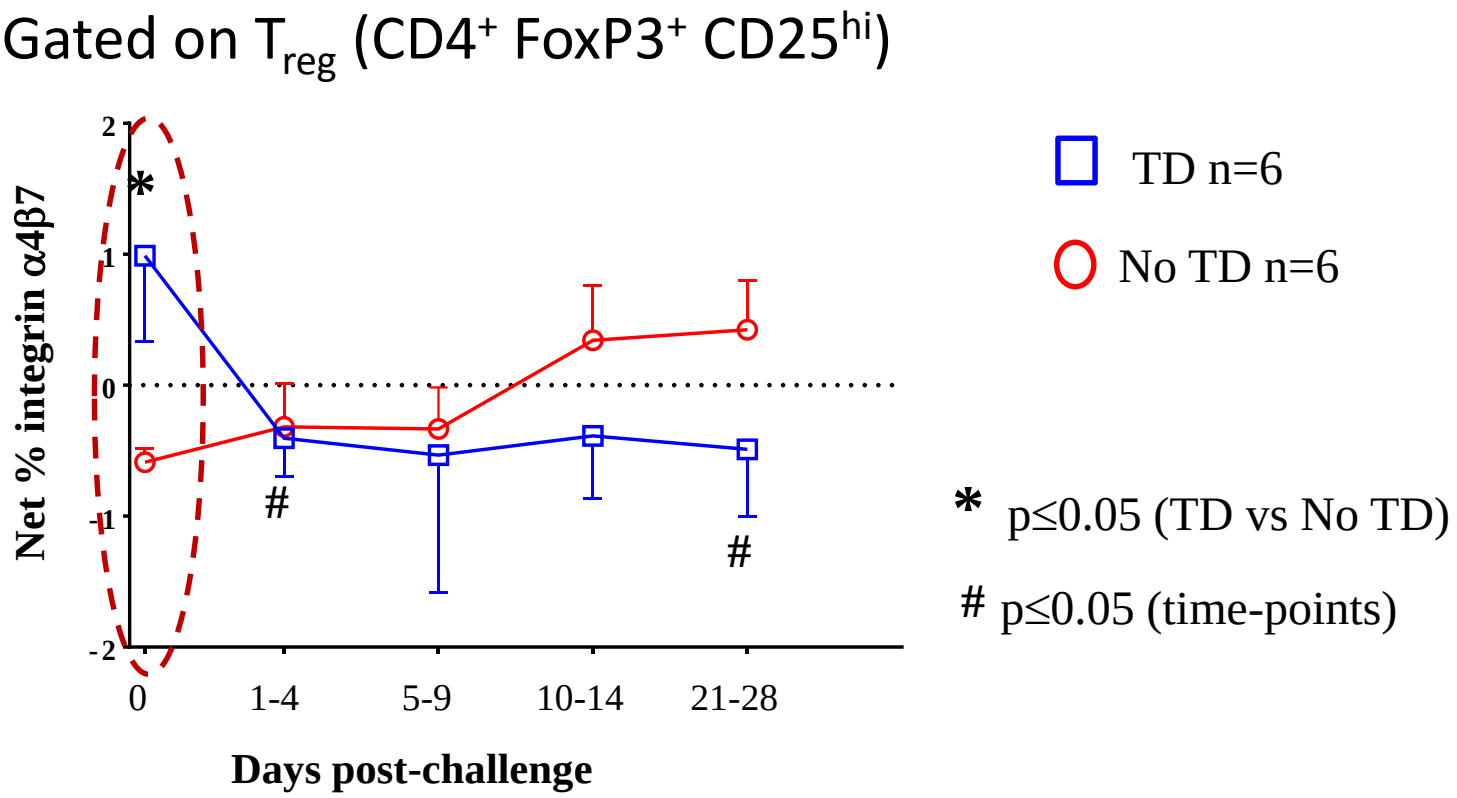
Gut homing of circulating *S.* Typhi-responsive T_{reg}



- ***S.* Typhi-responsive expression of integrin α4β7 is up-regulated on circulating Treg pre-challenge in TD volunteers indicating possible association with disease**
- **Down-regulation of *S.* Typhi-responsive integrin α4β7 expression on Treg post-challenge in TD volunteers indicates potential homing to the gut**

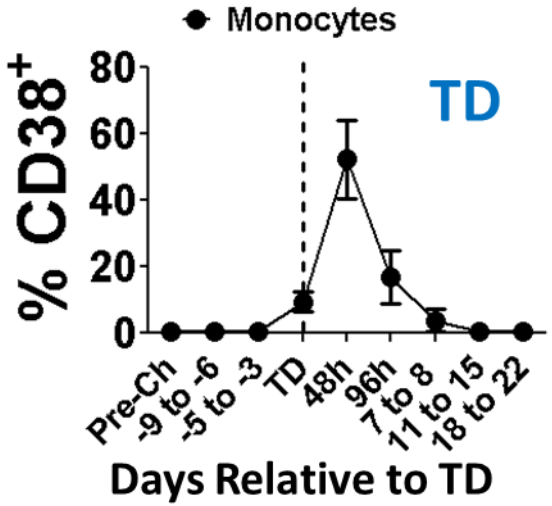
T regulatory cells: Conclusions

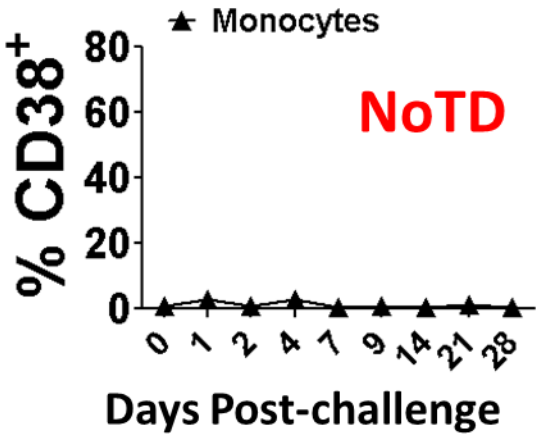
- *S.* Typhi-specific expression of integrin $\alpha 4\beta 7$ is up-regulated pre-challenge in TD volunteers
- *S.* Typhi-specific expression of activation molecules is increased in TD volunteers
- T_{reg} are capable of functionally suppressing *S.* Typhi-specific responses

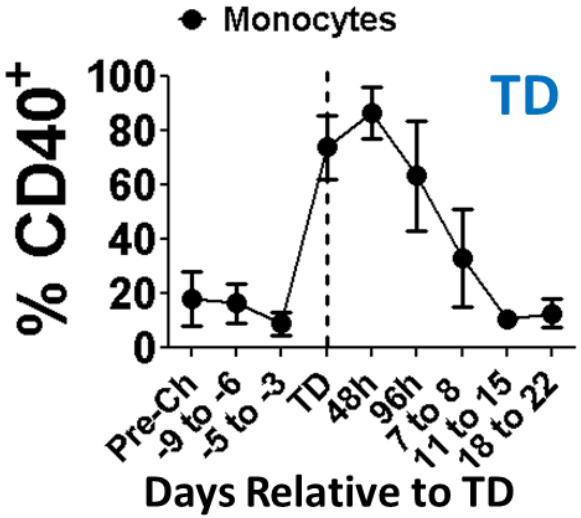
Oxford human wild-type *S.* Typhi challenge studies (Quailes strain)

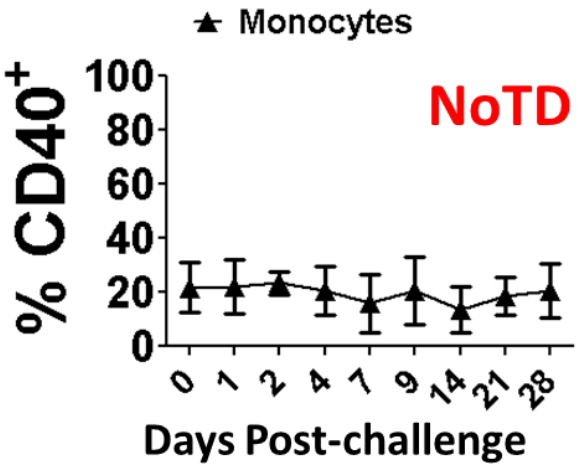
Antigen Presenting Cells (monocytes, dendritic cells)

Changes in CD38 and CD40 expression





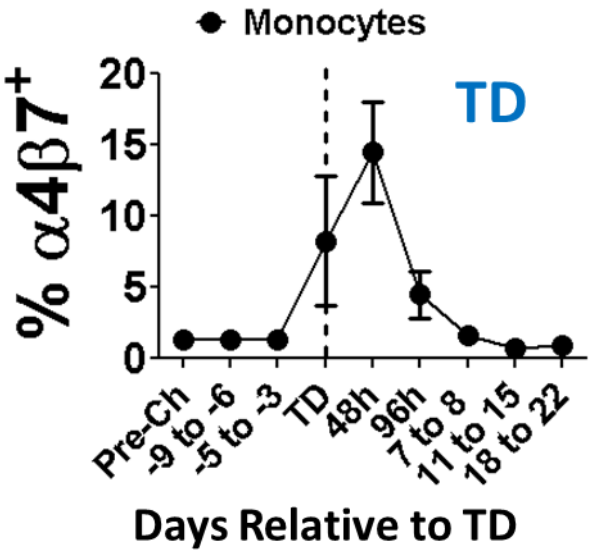


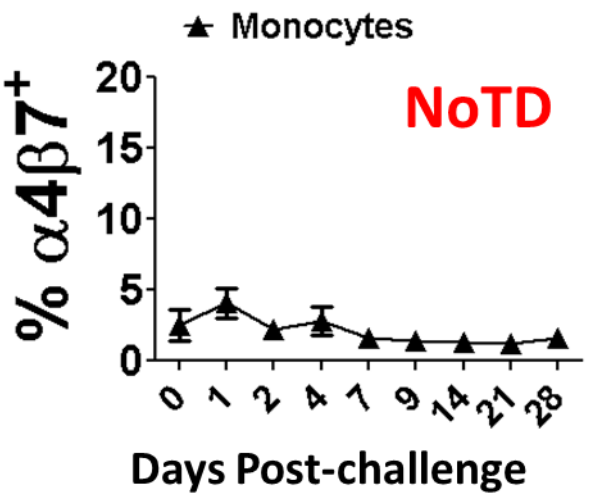


* p = 0.05; ** p = 0.005

CD38 and CD40 expression was up-regulated in monocytes only in TD subjects

Changes in integrin α4β7 expression

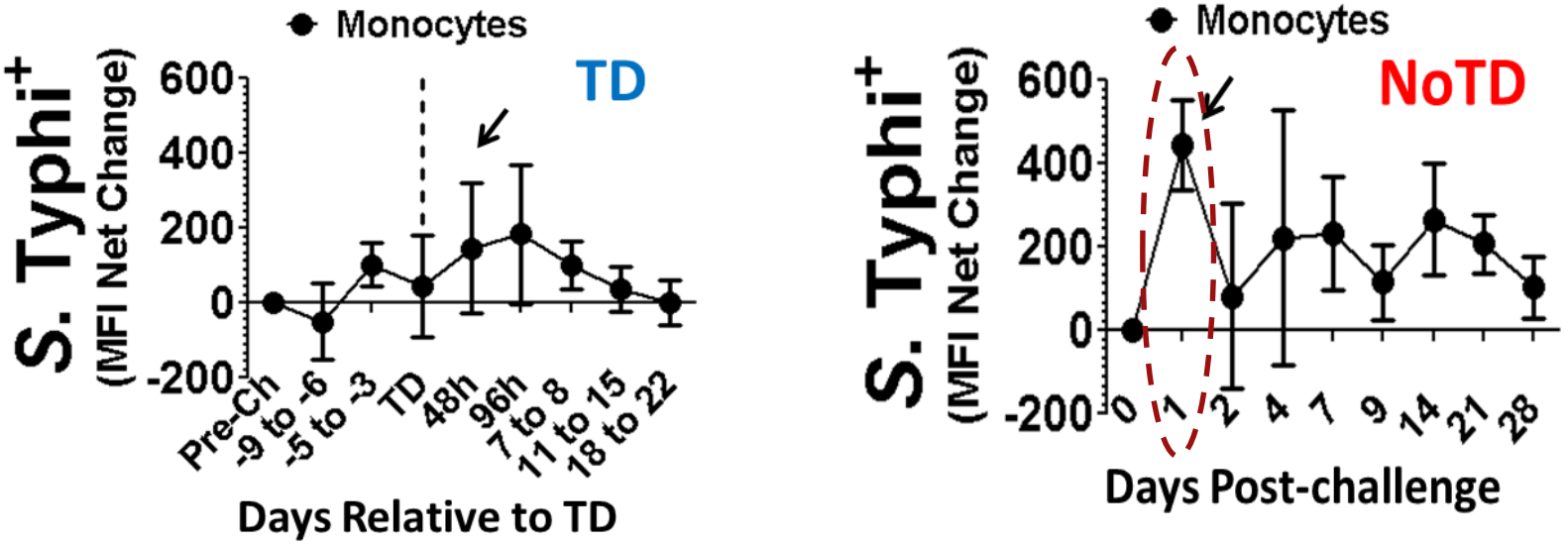




** p=0.005

Only monocytes isolated from TD volunteers showed up-regulation of integrin α4β7

Monocytes binding to *S.* Typhi



- In TD volunteers a spike in *S.* Typhi binding was detected during disease days (2 of 4 volunteers)
- In NoTD volunteers a spike in *S.* Typhi binding was also noted; however, it was present immediately after challenge (D1)

Fresnay et al. J Transl Med (2016) 14:62
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RESEARCH Open Access

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

Stephanie Fresnay[1], Monica A. McArthur[1], Laurence Magder[2], Thomas C. Darton[3], Claire Jones[3], Claire S. Waddington[3], Christoph J. Blohmke[3], Brian Angus[4], Myron M. Levine[1], Andrew J. Pollard[3] and Marcelo B. Sztein[1*]

PLOS | NEGLECTED TROPICAL DISEASES 2015

RESEARCH ARTICLE

Oral Wild-Type *Salmonella* Typhi Challenge Induces Activation of Circulating Monocytes and Dendritic Cells in Individuals Who Develop Typhoid Disease

Franklin R. Toapanta[1,2*], Paula J. Bernal[1,3], Stephanie Fresnay[1,3], Thomas C. Darton[4], Claire Jones[4], Claire S. Waddington[4], Christoph J. Blohmke[4], Gordon Dougan[5], Brian Angus[6], Myron M. Levine[1,2,3], Andrew J. Pollard[4], Marcelo B. Sztein[1,2,3*]

PLOS | PATHOGENS 2015

RESEARCH ARTICLE

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

Monica A. McArthur[1], Stephanie Fresnay[1], Laurence S. Magder[2], Thomas C. Darton[3], Claire Jones[3], Claire S. Waddington[3], Christoph J. Blohmke[3], Gordon Dougan[4], Brian Angus[5], Myron M. Levine[1], Andrew J. Pollard[3], Marcelo B. Sztein[1*]

PLOS | NEGLECTED TROPICAL DISEASES 2016

Oral Challenge with Wild-Type *Salmonella* Typhi Induces Distinct Changes in B Cell Subsets in Individuals Who Develop Typhoid Disease

Franklin R. Toapanta[1,2*], Paula J. Bernal[1,3], Stephanie Fresnay[1,3], Laurence S. Magder[4], Thomas C. Darton[5], Claire Jones[5], Claire S. Waddington[5], Christoph J. Blohmke[5], Brian Angus[6], Myron M. Levine[1,2,3], Andrew J. Pollard[5], Marcelo B. Sztein[1,2,3*]

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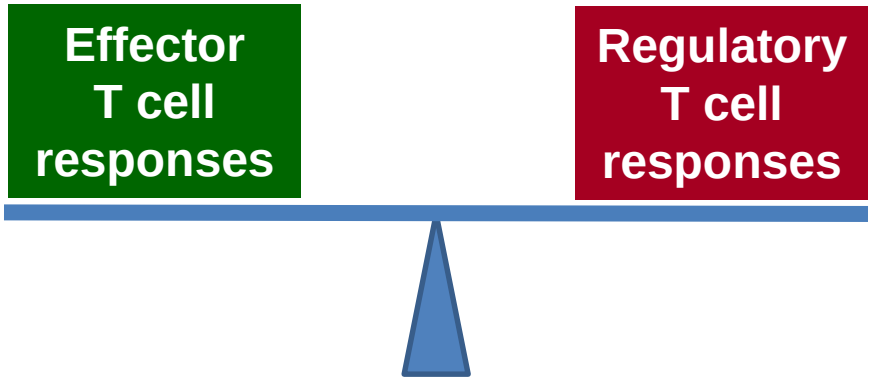
Importance of *Salmonella* Typhi-Responsive CD8+ T Cell Immunity in a Human Typhoid Fever Challenge Model

Stephanie Fresnay[1], Monica A. McArthur[1], Laurence S. Magder[2], Thomas C. Darton[3], Claire Jones[3], Claire S. Waddington[3], Christoph J. Blohmke[3], Brian Angus[4], Myron M. Levine[1], Andrew J. Pollard[3] and Marcelo B. Sztein[1]*

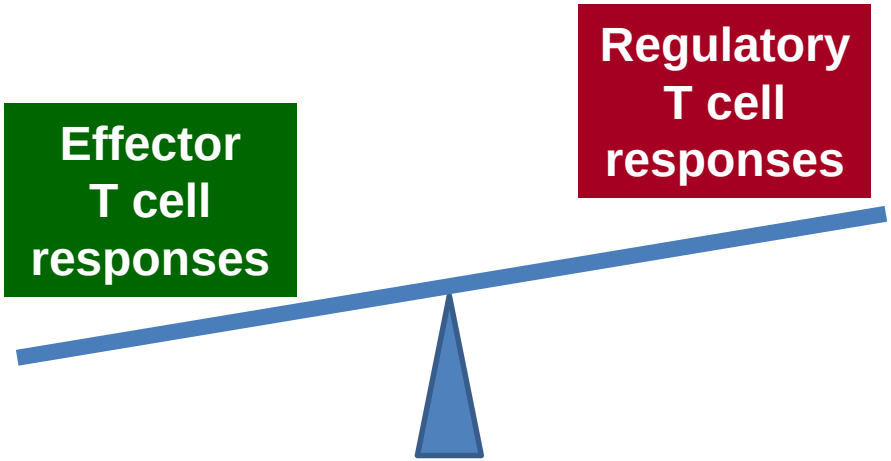
- A MAIT cell paper is In Press (Front. Immunol.)
- Correlation of all data to evaluate whether a single response can be a CoP **(best case scenario)** or a combination of immune responses is more indicative of short and long-term protection than individual responses

CVD

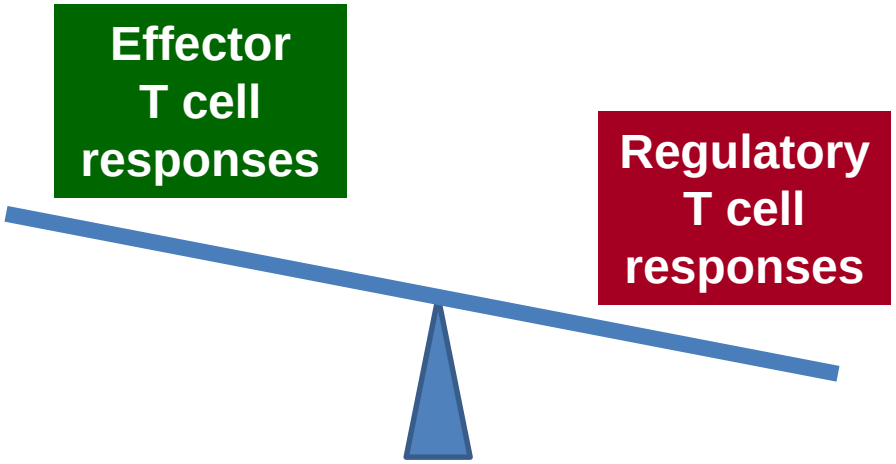
Balancing the immune response



Homeostasis (protection?)

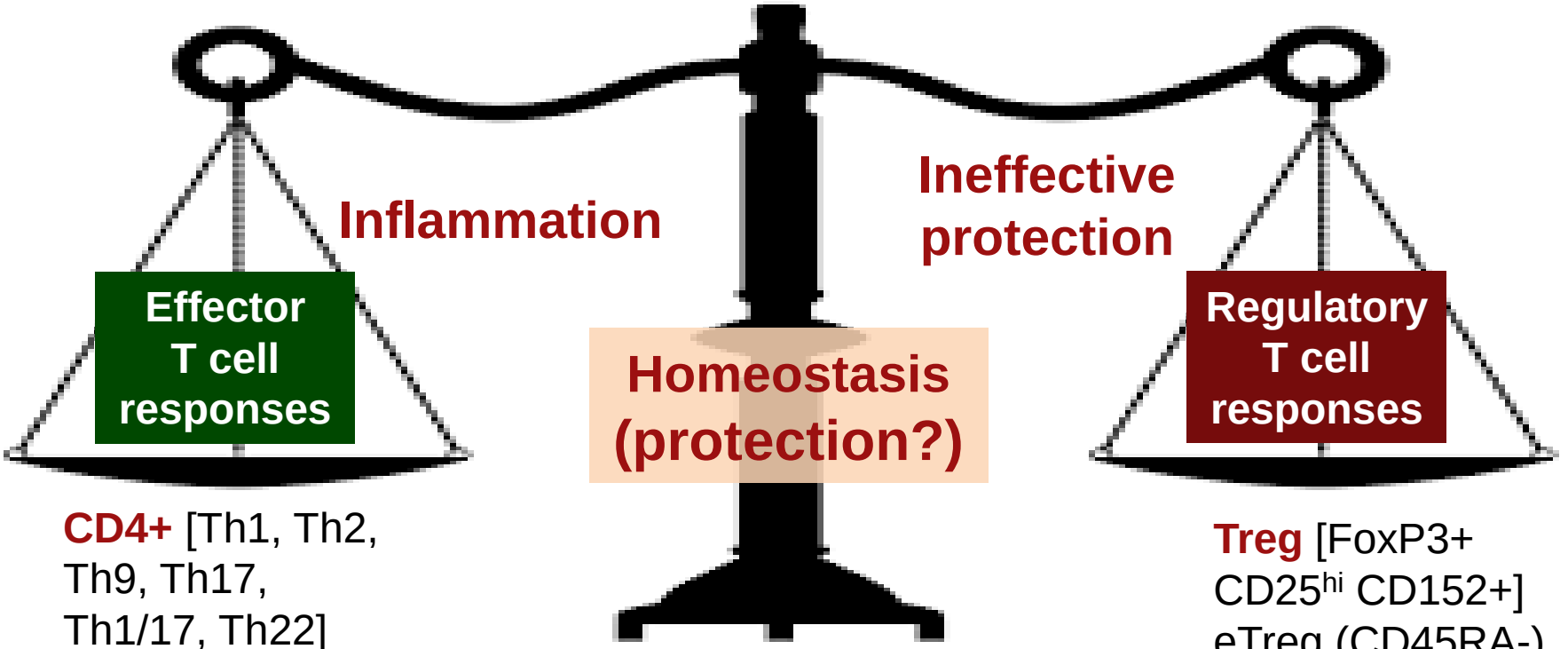


Ineffective protection



Inflammation

Balancing the immune response



CD4+ [Th1, Th2, Th9, Th17, Th1/17, Th22]

CD8+ [Tc1, Tc2, CTL, conv Fox-P3^{dim} T, others]

MF, which concomitant cytokines?

Humoral immunity

APC (Mϕ, DC, B) & other cells that bridge adaptive and innate immunity (e.g., **MAIT**, Tγδ)

Treg [FoxP3+ CD25^{hi} CD152+] eTreg (CD45RA-), nTreg (CD45RA+), Tr1, Tr3, Th3, Th1/17-10, others

MF, which concomitant cytokines?

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

Overall Conclusion

Identifying 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 new generations of more effective vaccines against infectious diseases

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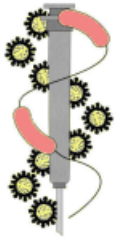
CVD



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Stephen Lockhart

Clinical studies

Myron Levine

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