

New insights into the interplay between *Shigella* and human lymphocytes

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ECMIS 2019
2-5 June: Het Pand – Ghent University

Shigella - causal agent of bacillary dysentery

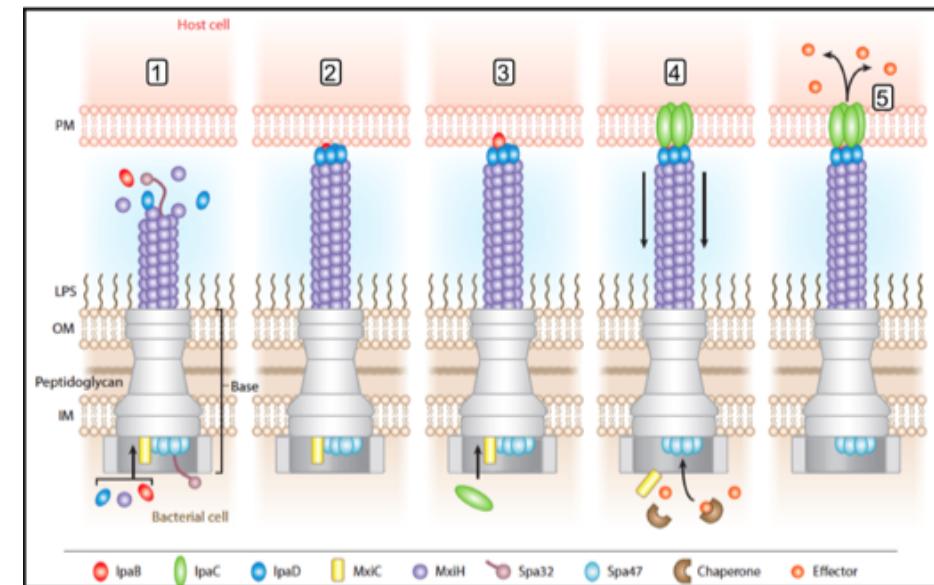
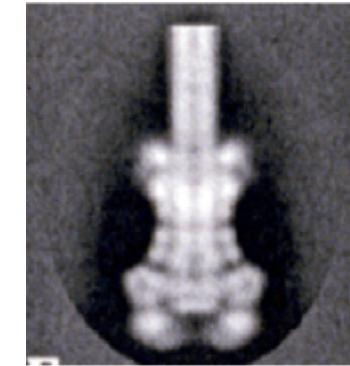
- Enteroinvasive bacterium
- Highly infective: up to 100 bacteria in human
- Dysentery with fever, intestinal cramps and mucoid bloody stools
- Invasion, dissemination of human colonic mucosa
 - Neutrophil (PMN) infiltration
 - Massive tissue destruction
 - Excessive cell death
- Children younger than 5 years in less-developed countries



Kosek M. et al. 2003, Bull. WHO
Von Seidlein L. et al. 2006, PLoS Medicine

Shigella Type Three Secretion System (T3SS)

- Determines bacterial virulence
- Essential for cell invasion
- T3SS proteins encoded on the virulence plasmid
- Functionality:
 - Part of T3S apparatus (MxiD)
 - Needle tip effectors (IpaD)
 - Injection effectors (IpgD, VirA, IpaJ)



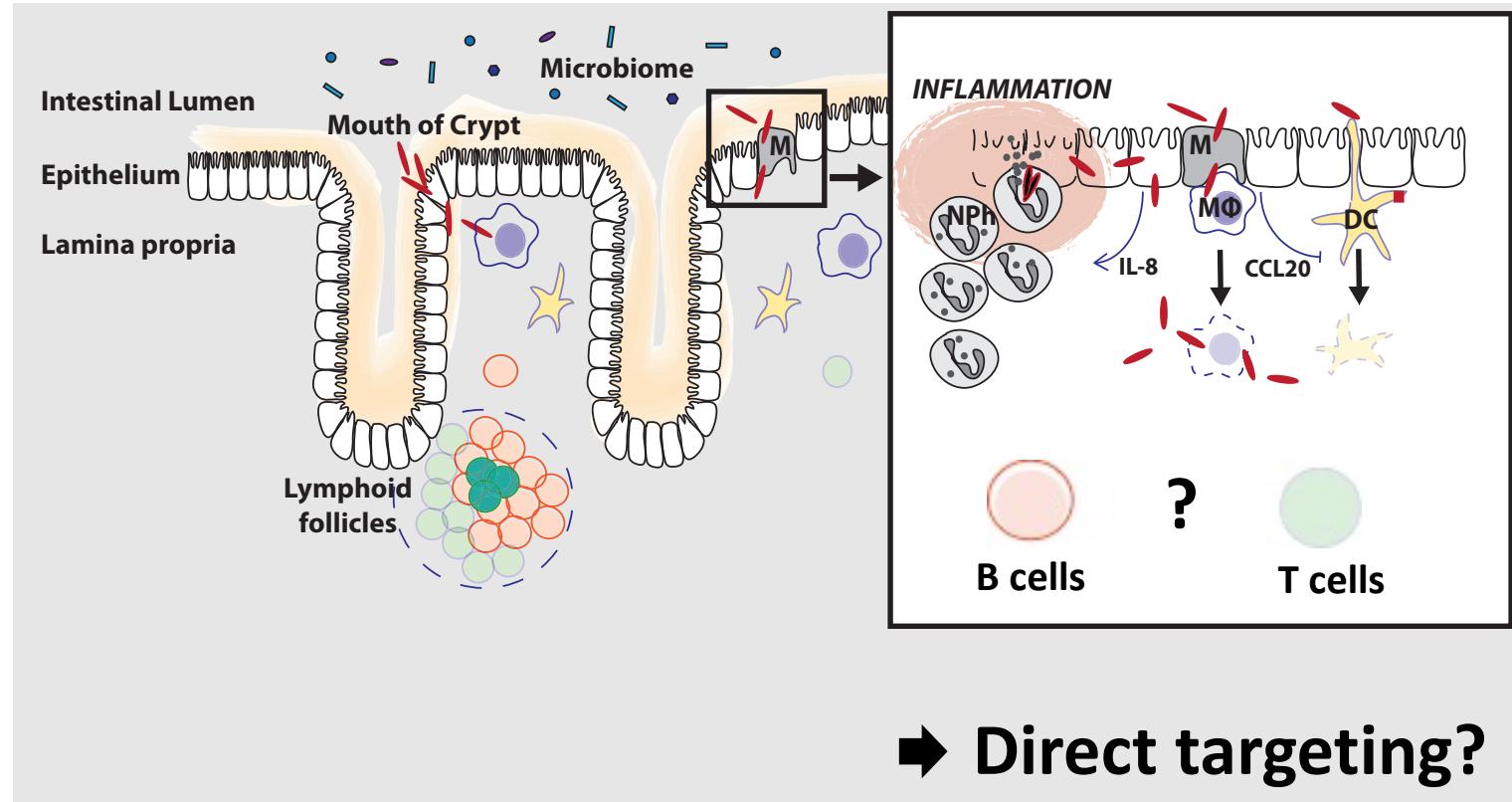
Hayes et al. 2010, Ann Rev Gen

Lack of long-term protective immunity

- Several infections required to mount a humoral protective response of short duration
- **Impairment of the priming of the specific response?**

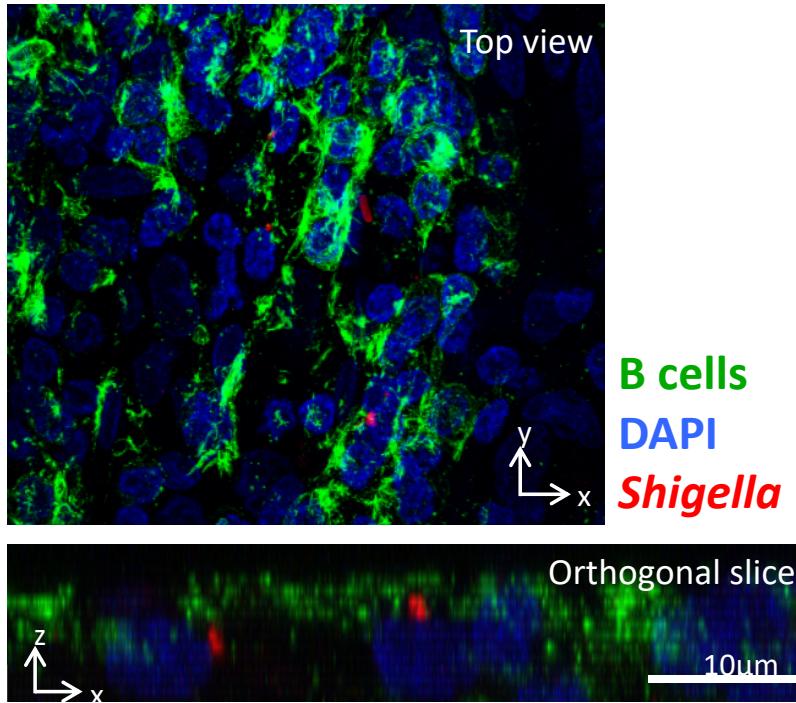
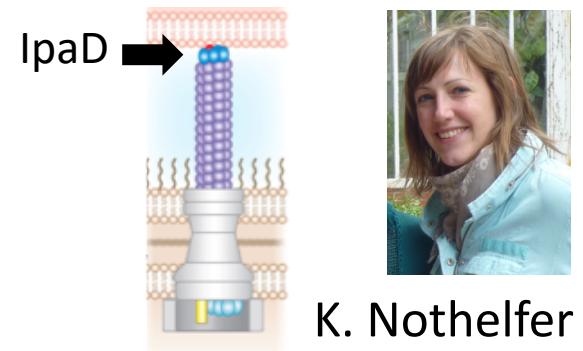
→ Acute inflammation?

- Invasion/dissemination of epithelium
- Activation of Innate immunity
- Infiltration of PMN
- Massive cell death
- Reduced DC recruitment

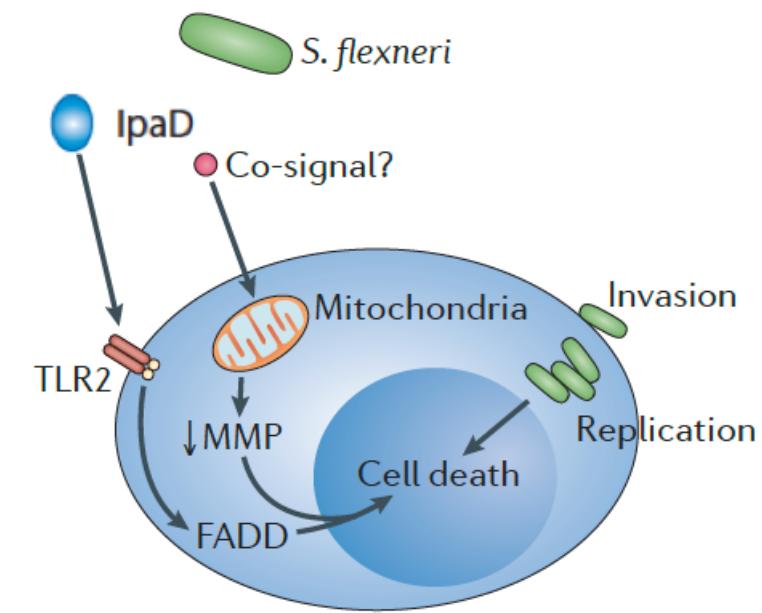
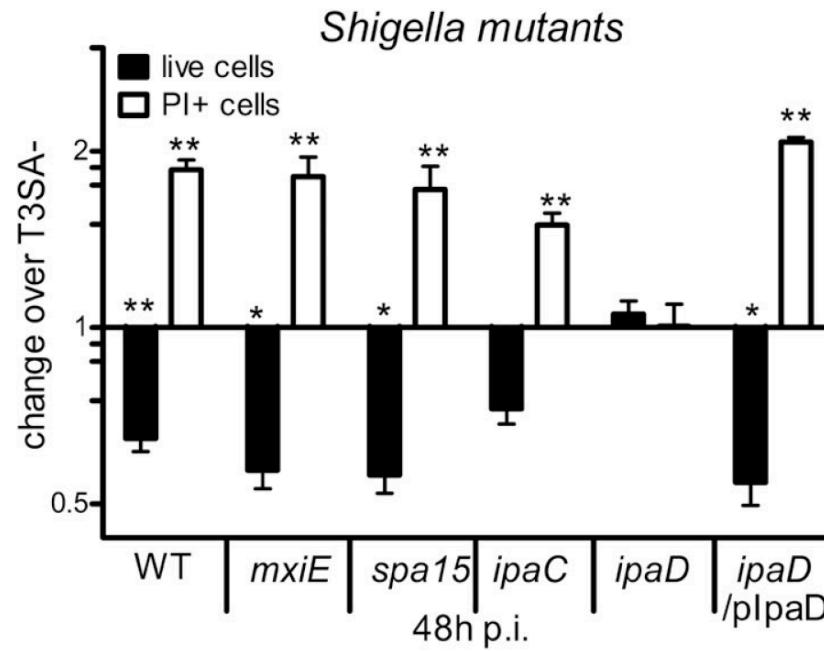


→ Direct targeting?

B lymphocyte targeting by *Shigella*



human colonic tissue/ *ex vivo* infection



→ B cell death by necrosis and apoptosis

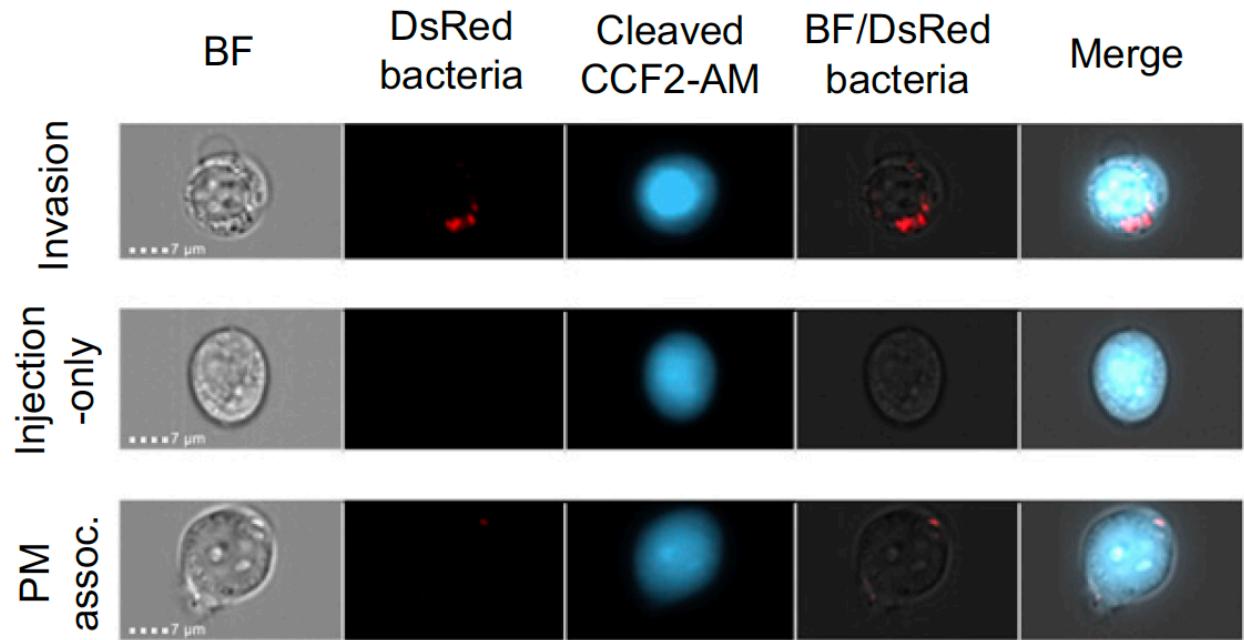
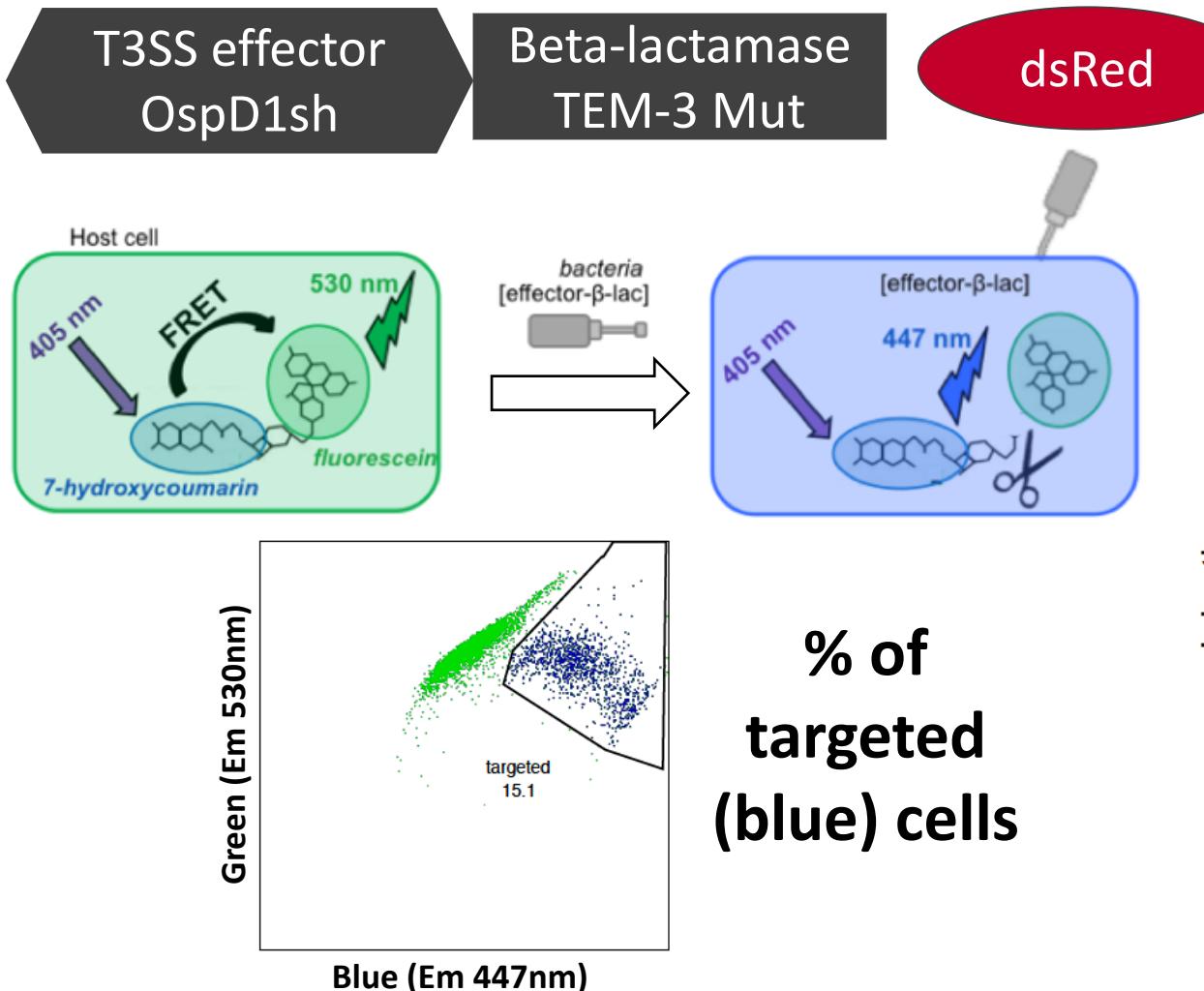
→ Invasion independent action of *Shigella*

Nothelfer K. et al., 2014, *J. Ex. Med.*
Nothelfer K. et al., 2015, *Nature Rev. Microbiol*
Hayes et al., 2010, *Ann Rev Gen*

Shigella reporter strain to monitor cell targeting



L. Pinaud

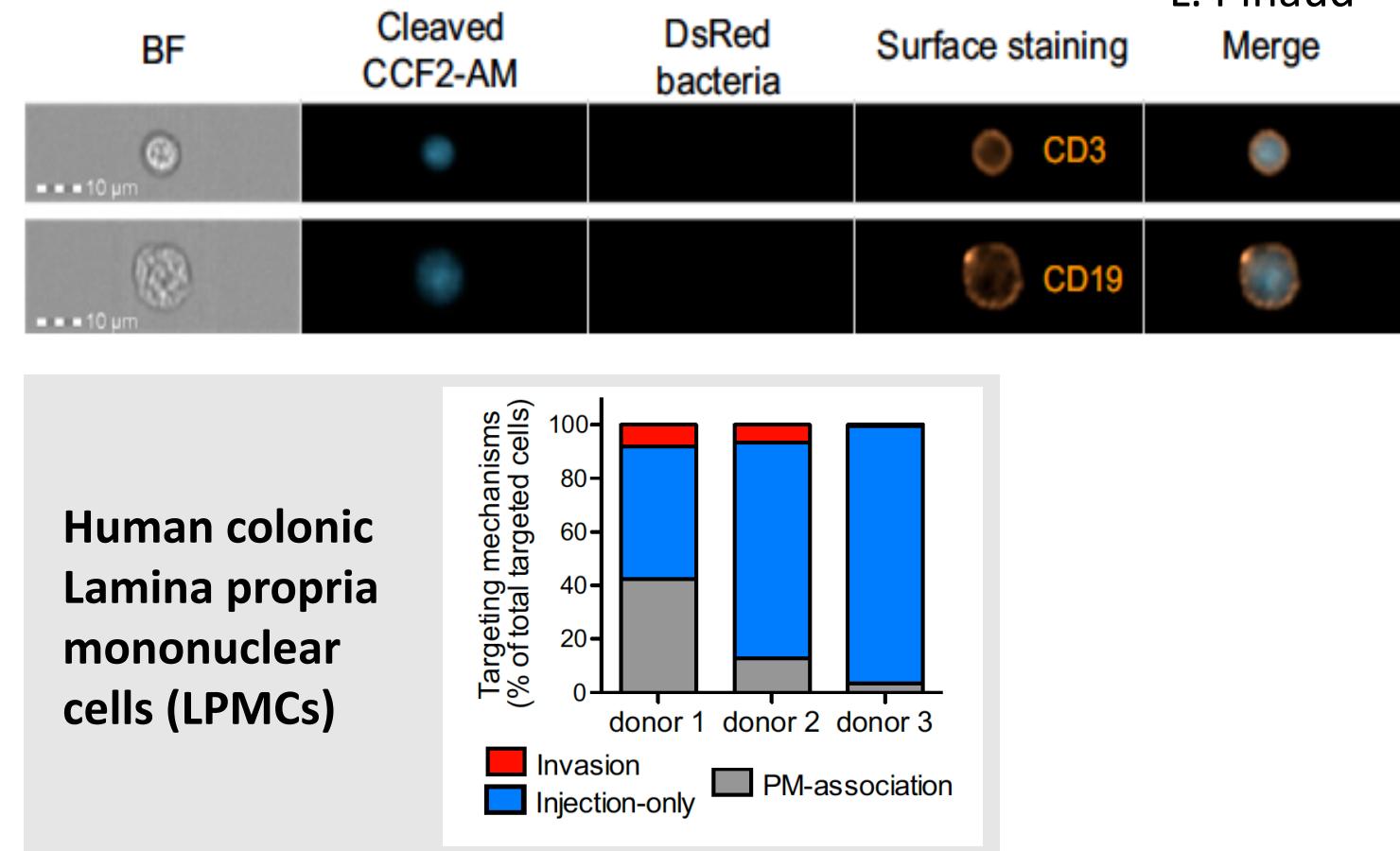
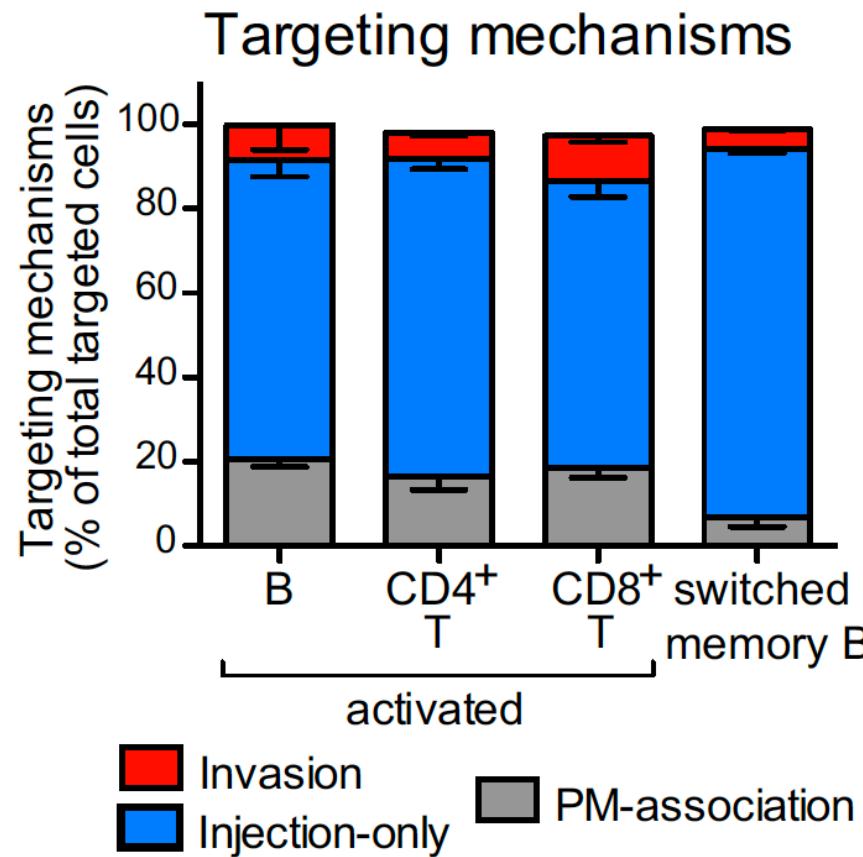


Pinaud L. et al. PNAS, 2017

Lymphocytes primarily targeted via injection-only



L. Pinaud
Merge



Pinaud L. et al. PNAS, 2017

Shigella targeting only in activated lymphocytes

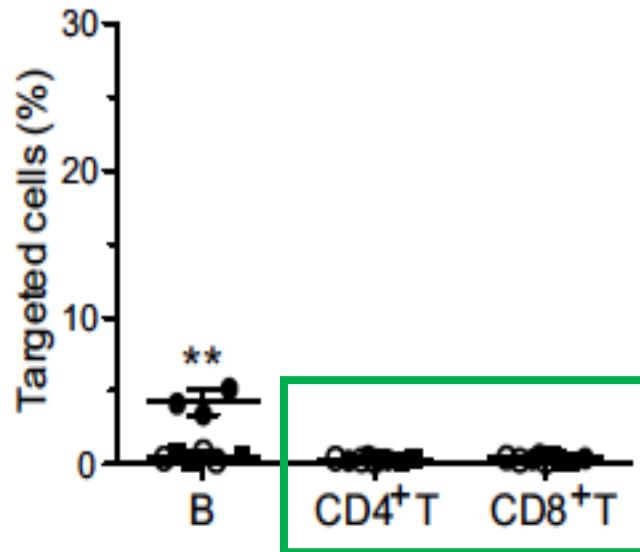


L. Pinaud

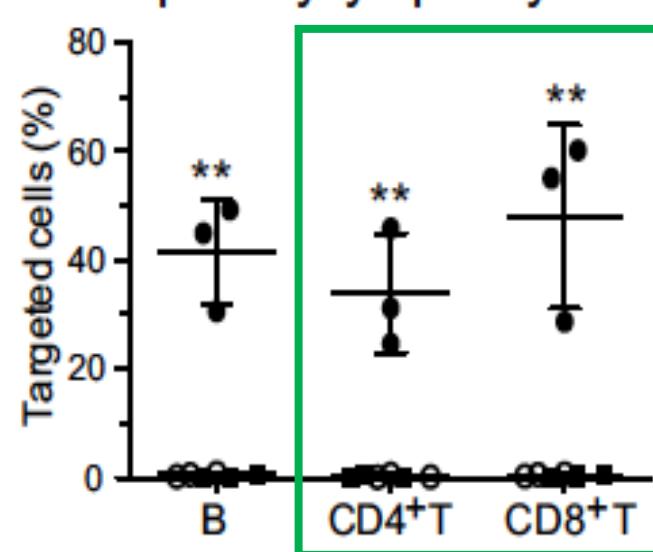


C. Konradt

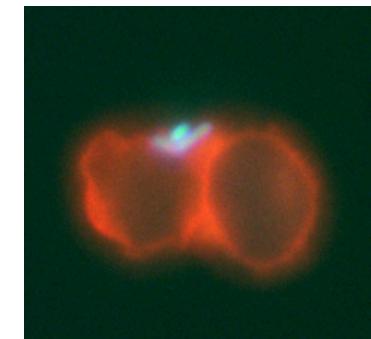
Non-activated blood primary lymphocytes



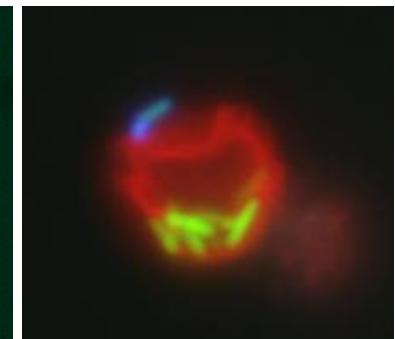
Activated blood primary lymphocytes



non-activated



activated



Pinaud L. et al. PNAS, 2017

Konradt C. et al. 2011, Cell Host Microbe



Institut Pasteur

Katja Brunner, 3. June 2019

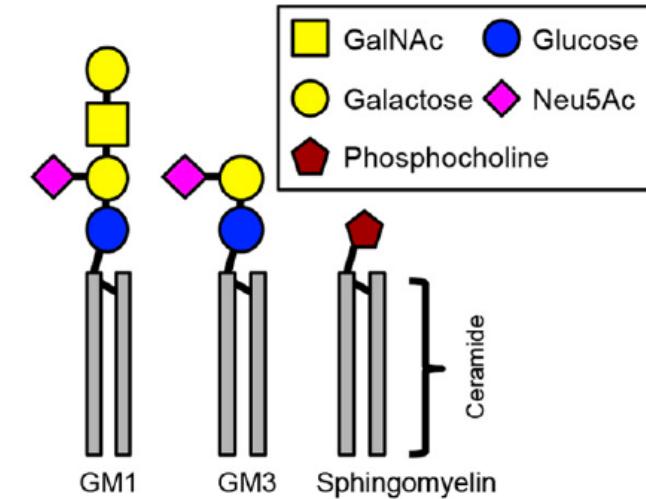
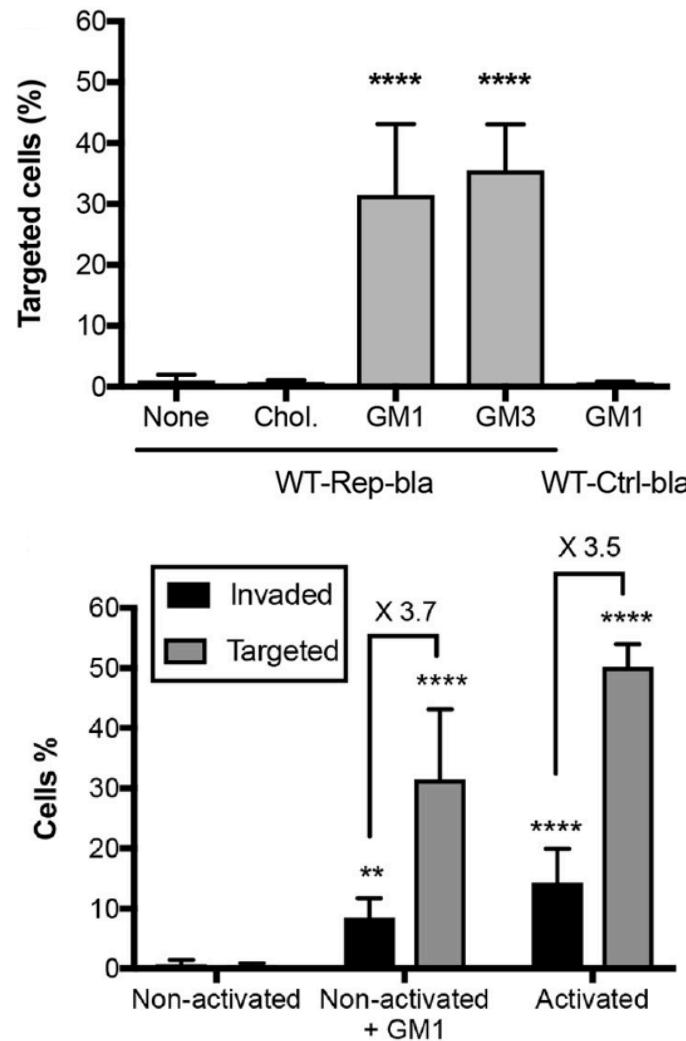
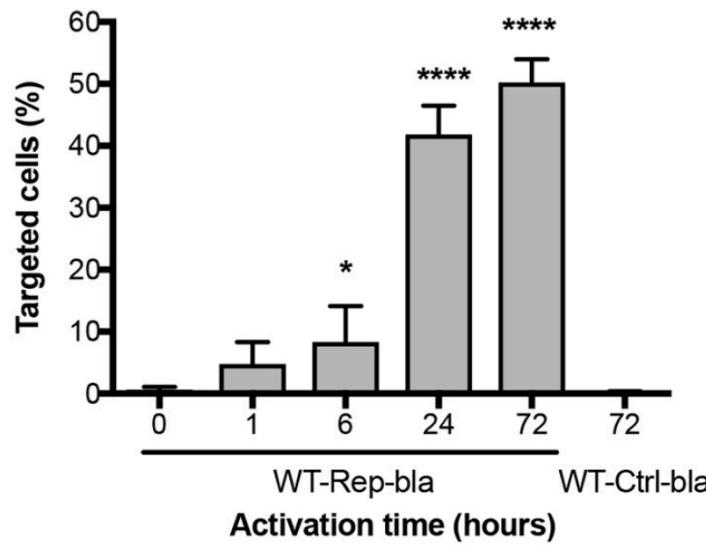
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Surface glycans important for T cell targeting



I. Belotserkovsky



**Sialated
glycosphingolipids
(gangliosides)**

Belotserkovsky I. et al. 2018, *mBio*

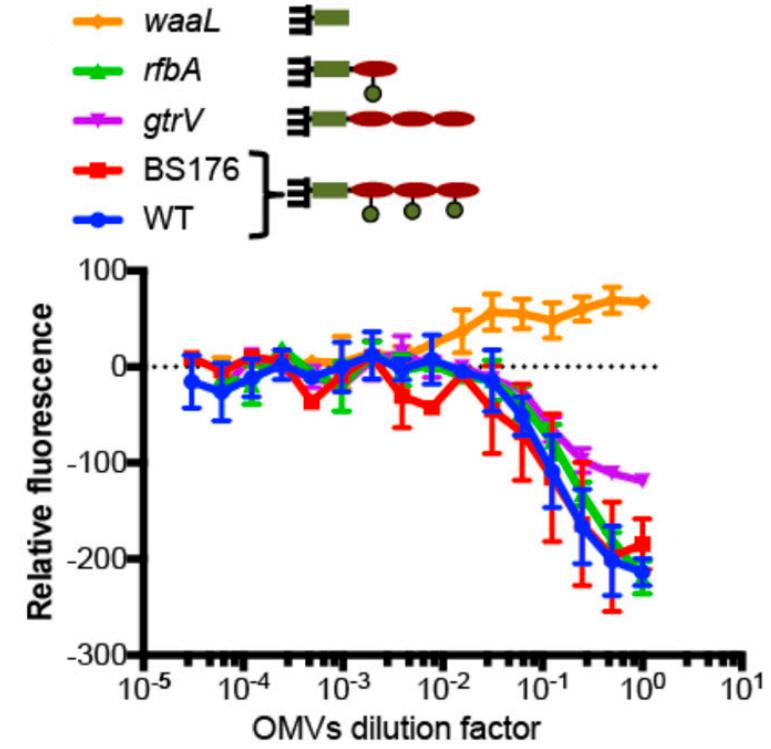
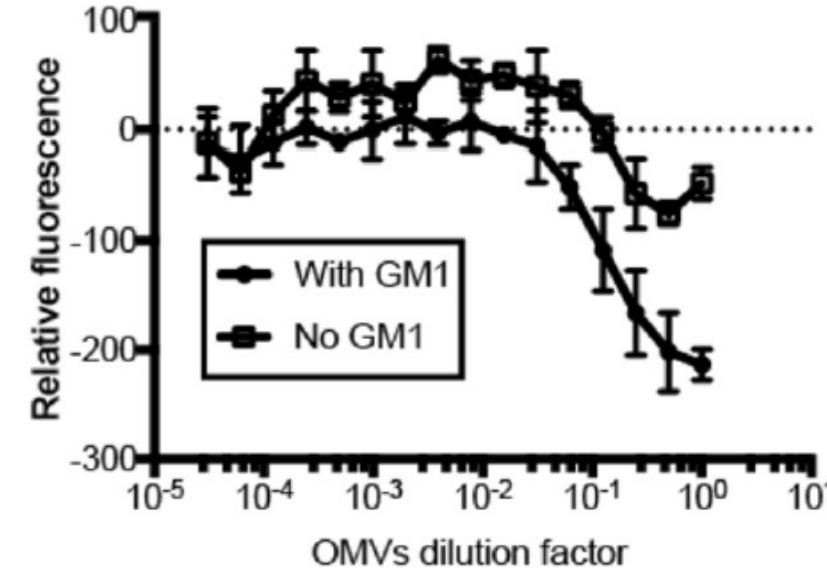
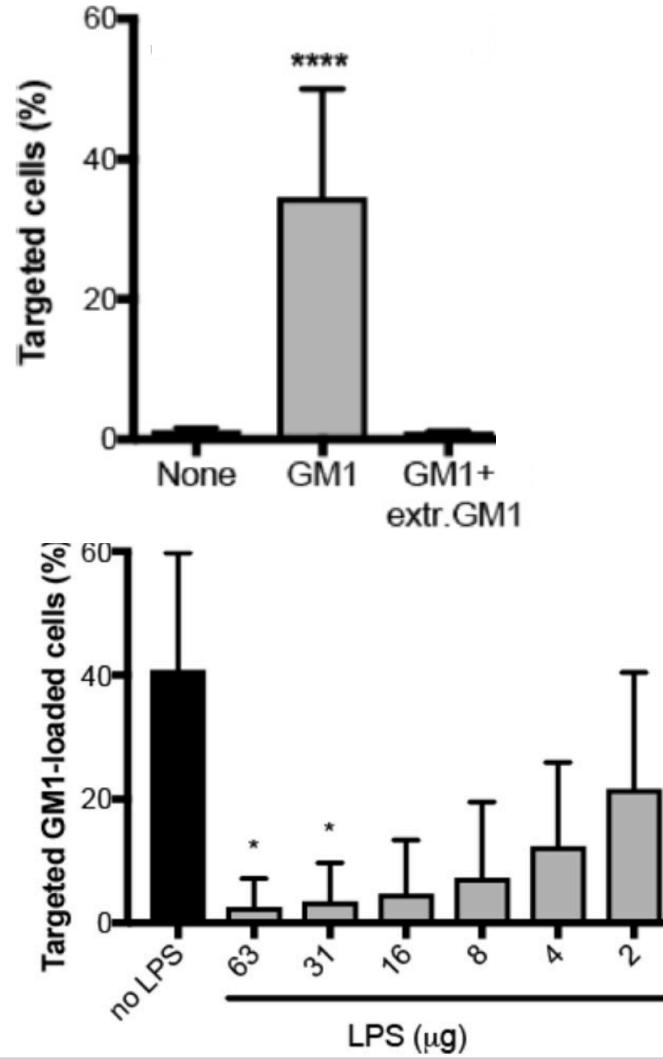
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GM1 promotes binding via glycan-glycan interaction

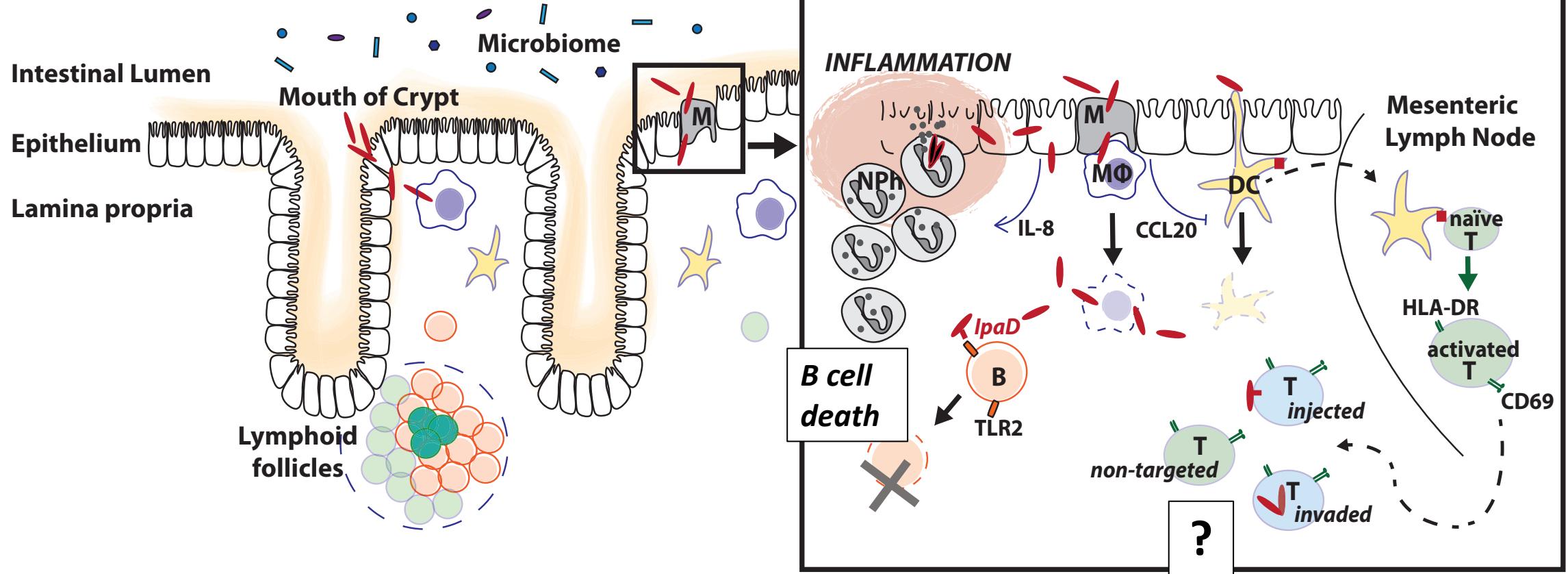


I. Belotserkovsky



Belotserkovsky I. et al. 2018, *mBio*

Direct targeting of lymphocytes



→ Functional consequences?

Brunner et al. 2019, Hum Vaccin Immunother

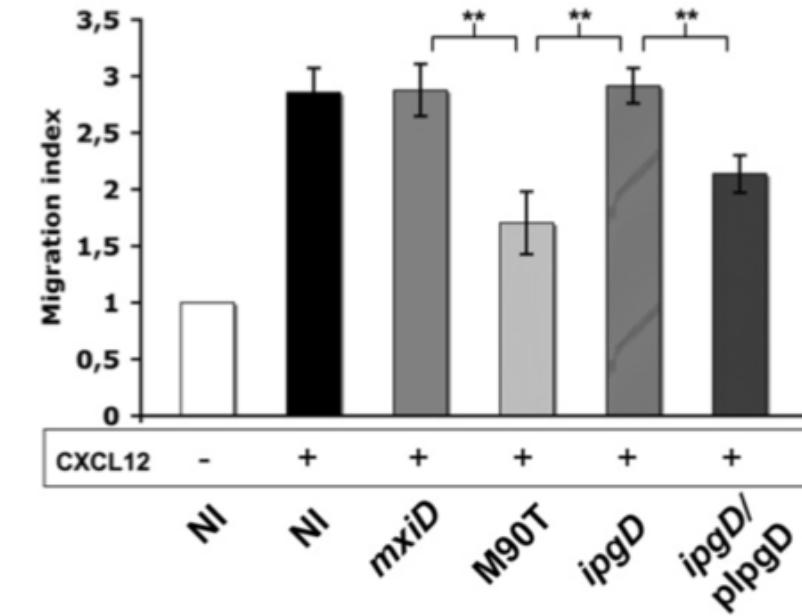
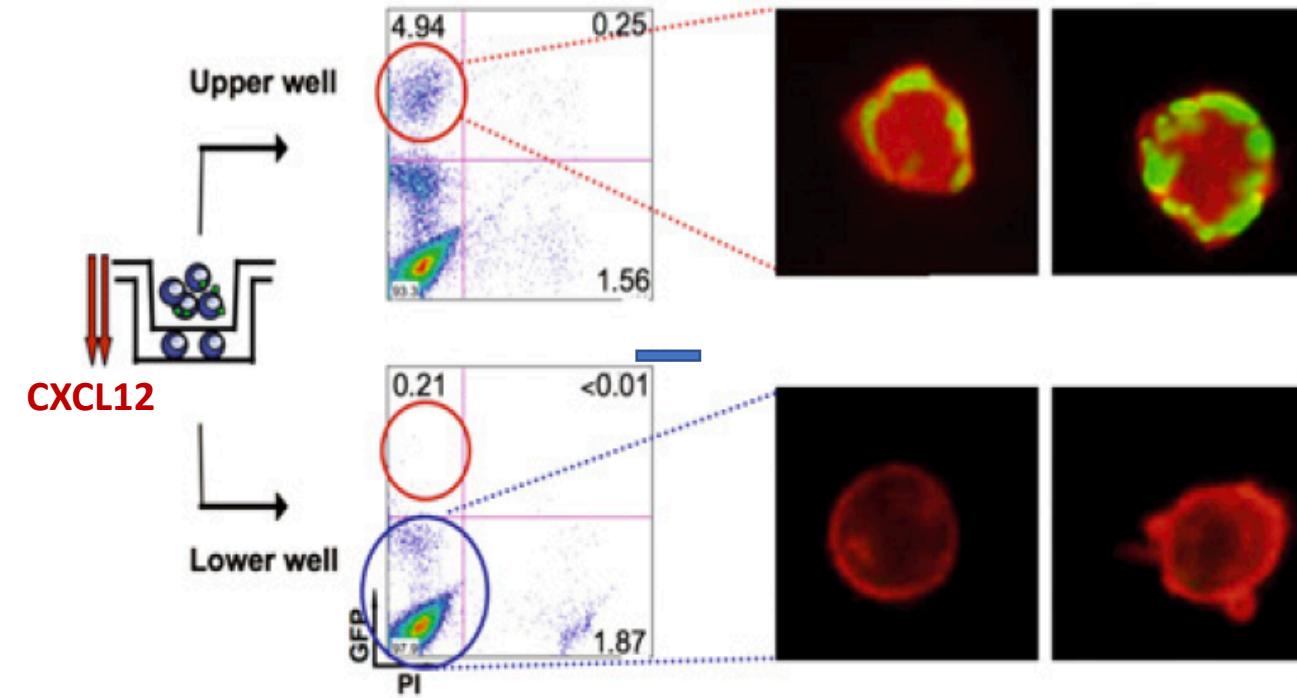
Shigella inhibits T cell chemotaxis



C. Konradt



E. Frigimelica



→ IpgD mediates hydrolysis of PIP₂ membrane pool

Konradt C. et al. 2011, Cell Host and Microbe

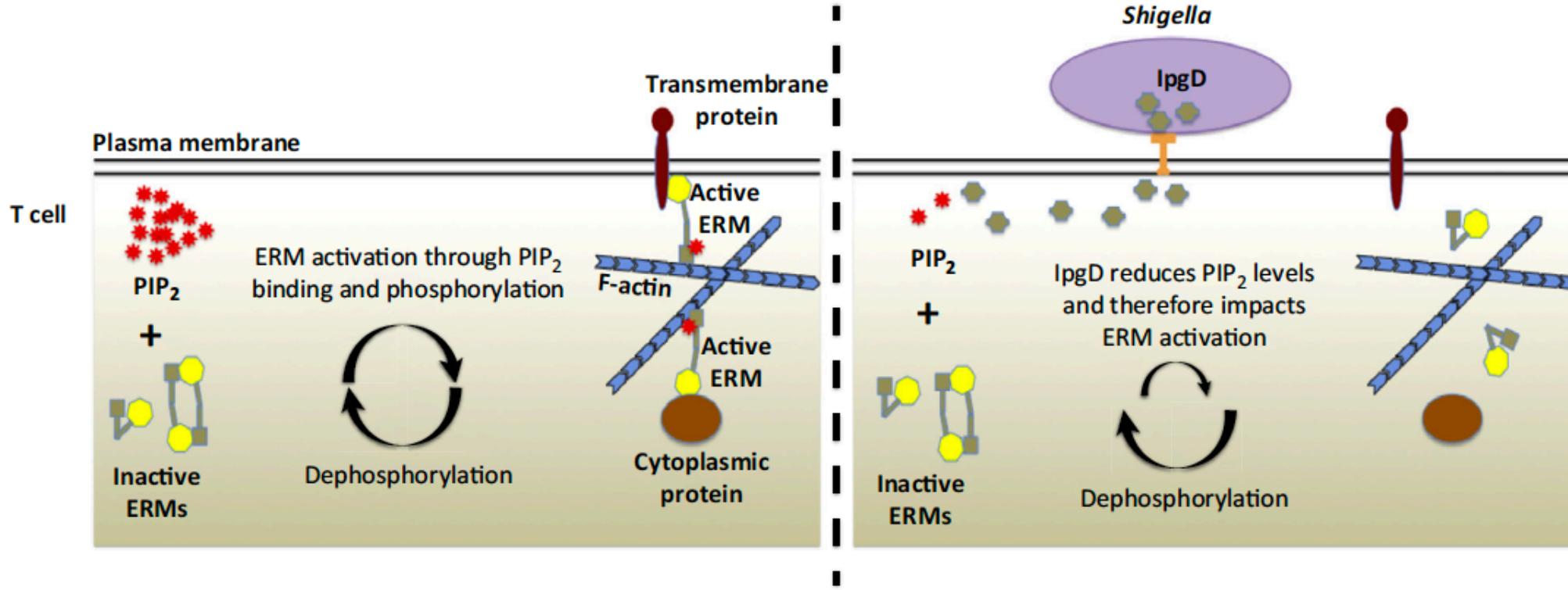


Shigella effector IpgD impairs cell polarisation

C. Konradt



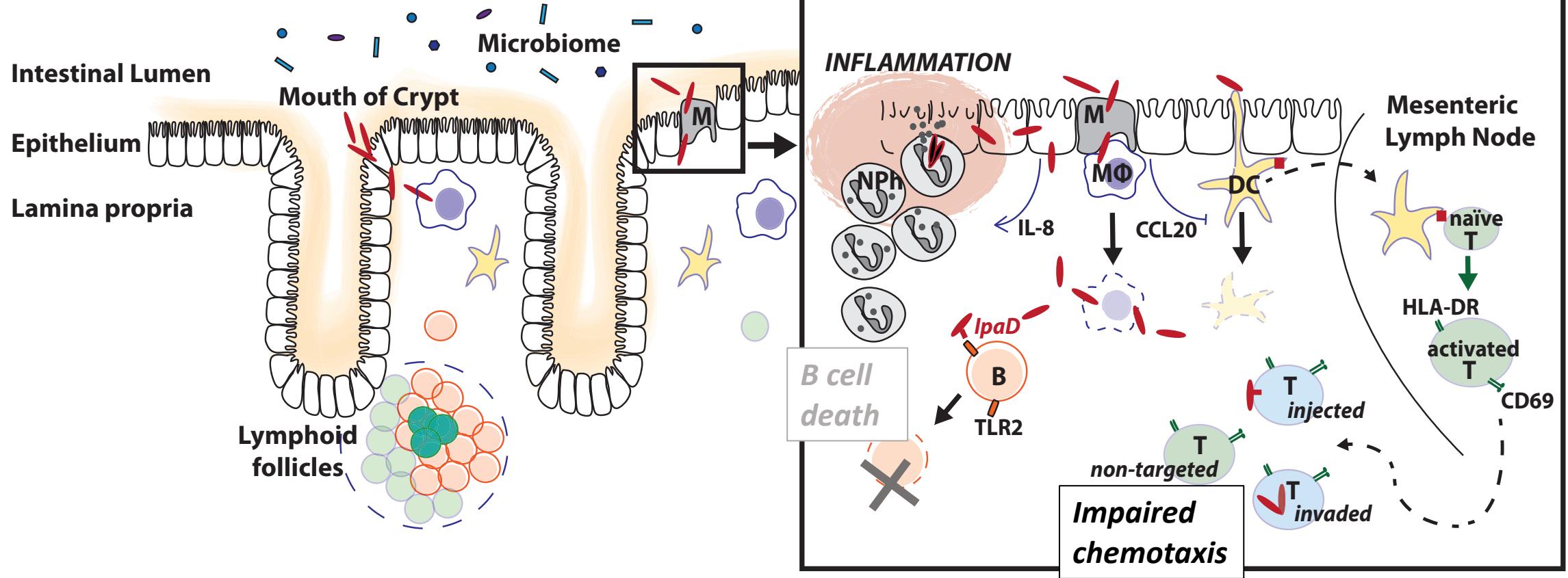
E. Frigimelica



→ IpgD-mediated PIP₂ pool & ERM phosphorylation

Konradt C. et al. 2011 *Cell Host and Microbe*
Salgado-Pabon W. et al. 2014 *Trends in Microbiology*

Direct targeting of lymphocytes

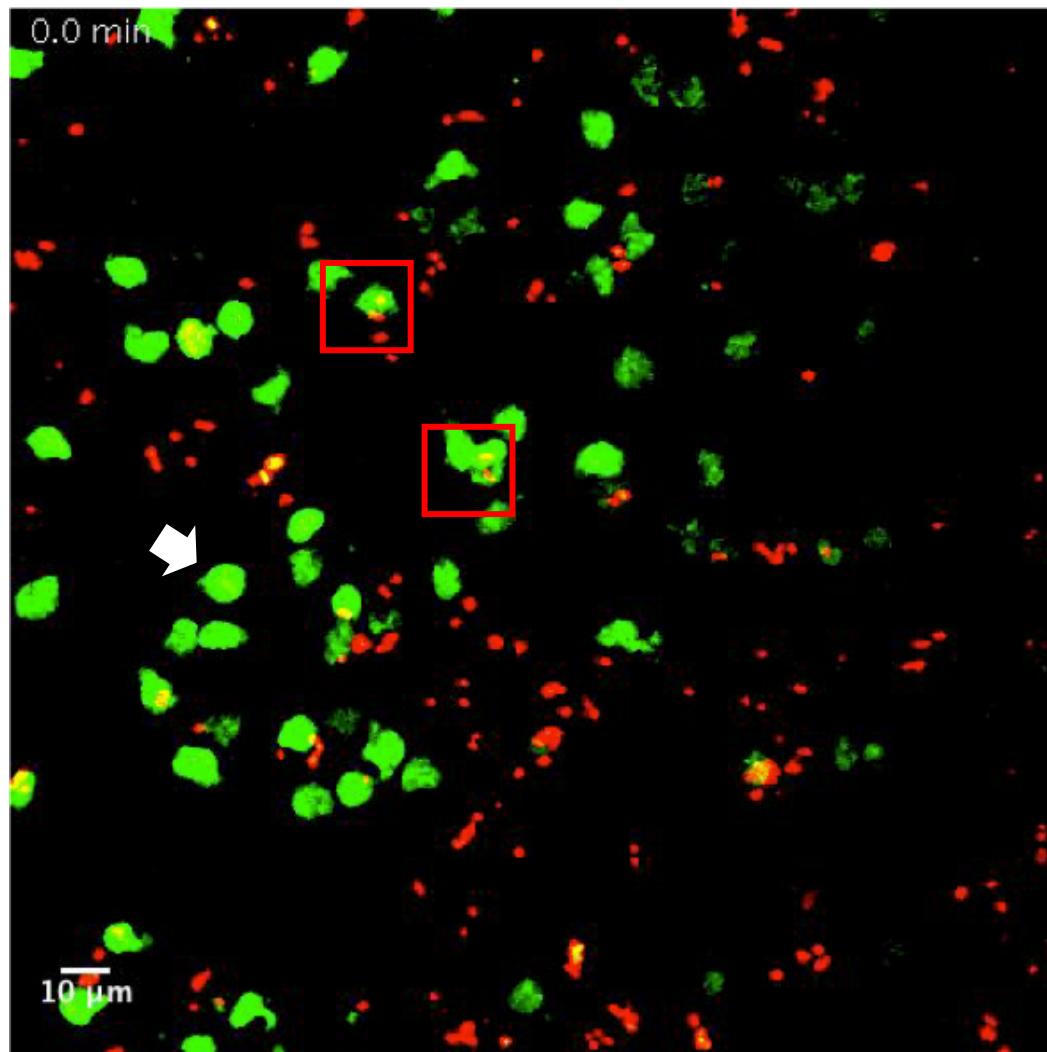


Brunner et al. 2019, Hum Vaccin Immunother

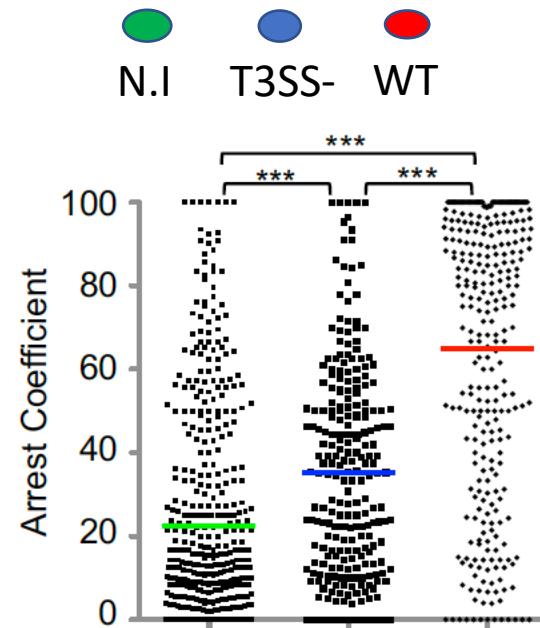
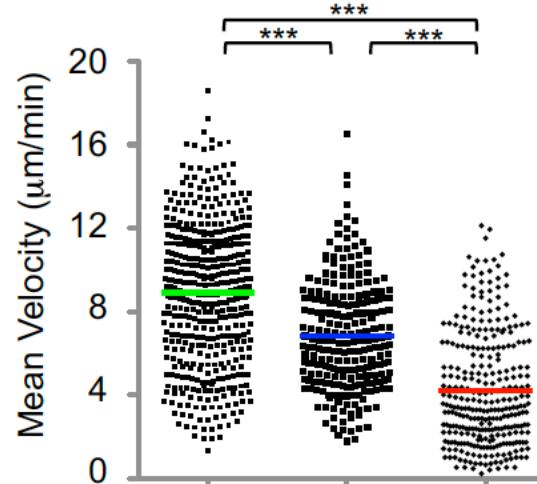
Impaired T cell mobility *in vivo*



W. Salgado-Pabon



Live imaging/
poplietal LN
upon footpad
injection



Salgado-Pabon W. et al 2013, PNAS

Immune synapse formation

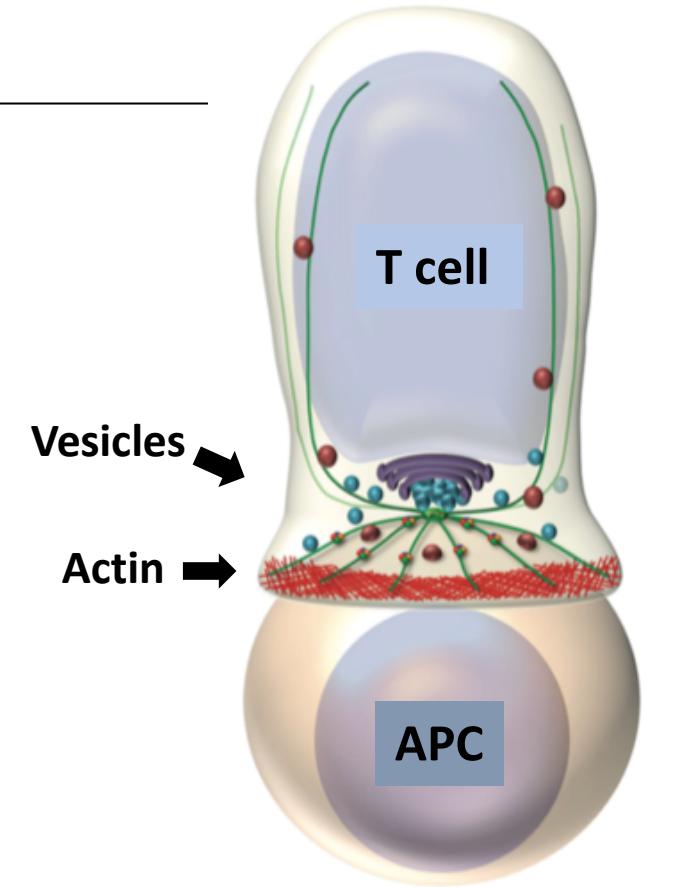
- Immune synapse formation leads to T cell activation
- **Mobility crucial for T cell scanning ability for Antigen presenting cells (APCs) and form conjugates**

➔ Does *Shigella* reduce conjugate formation?

- Canonical IS formation dependent on 2 main factors

1. Actin rearrangement
2. Vesicular trafficking

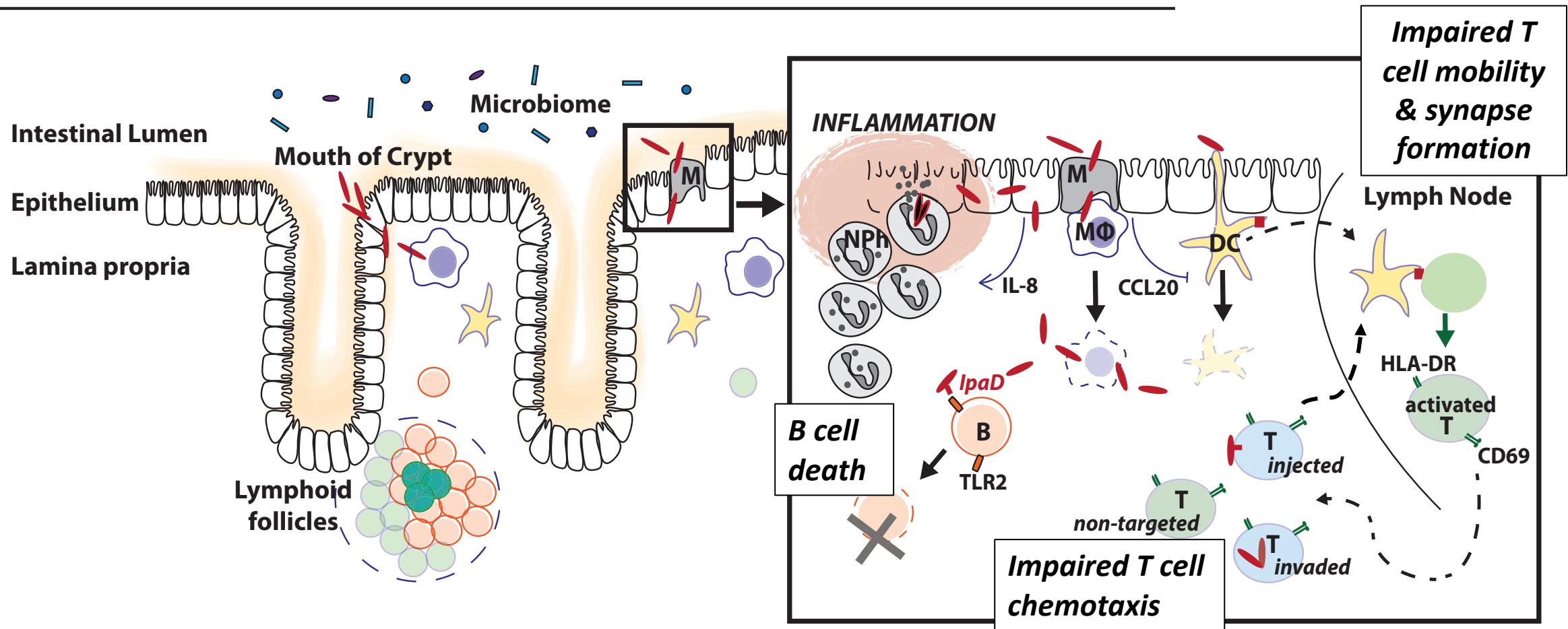
➔ Does *Shigella* impact canonical IS formation?



Immunological
synapse (IS)

Agüera-Gonzalez S. et al, 2015, eLS

Direct targeting of lymphocytes



Brunner et al. 2019, Hum Vaccin Immunother

Summary: *Shigella* and human lymphocytes

DIRECT TARGETING

- *Shigella* targets B and T lymphocytes
- Targeting is dependent on glycan-glycan interactions
- Injection-only phenotype suggestive for invasion independent action
***new paradigm in *Shigella* pathogenicity**

FUNCTIONAL IMPACT

- B cell death
- Impaired T cell chemotaxis and IS formation

- ➔ Contribution to inefficient priming of adaptive immunity?
- ➔ Involvement of other cell types?
- ➔ Cellular specificity of targeting in *lamina propria*?

Preferential targeting by *Shigella* using CyTOF technology

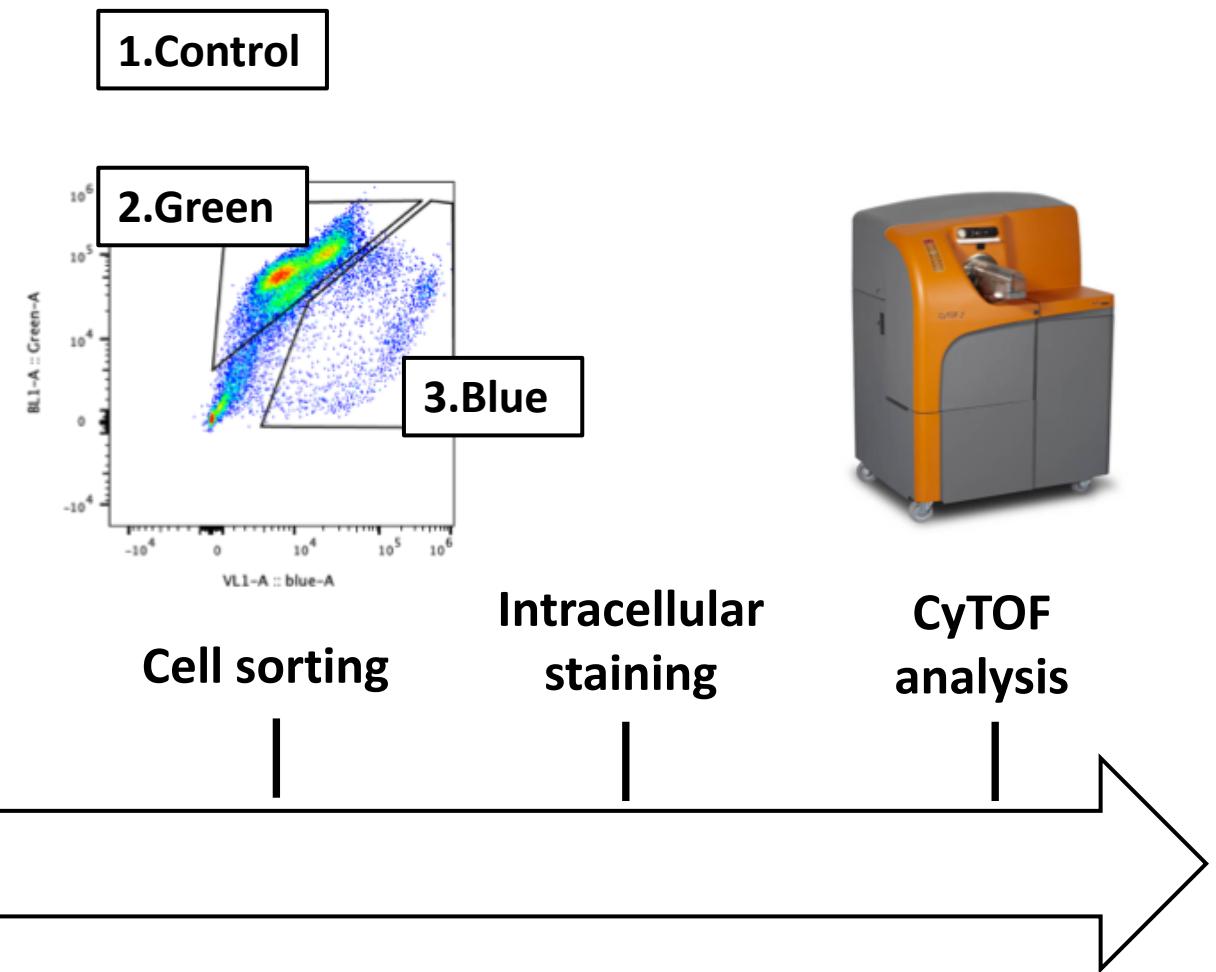


PBMCs
OR
LPMCs

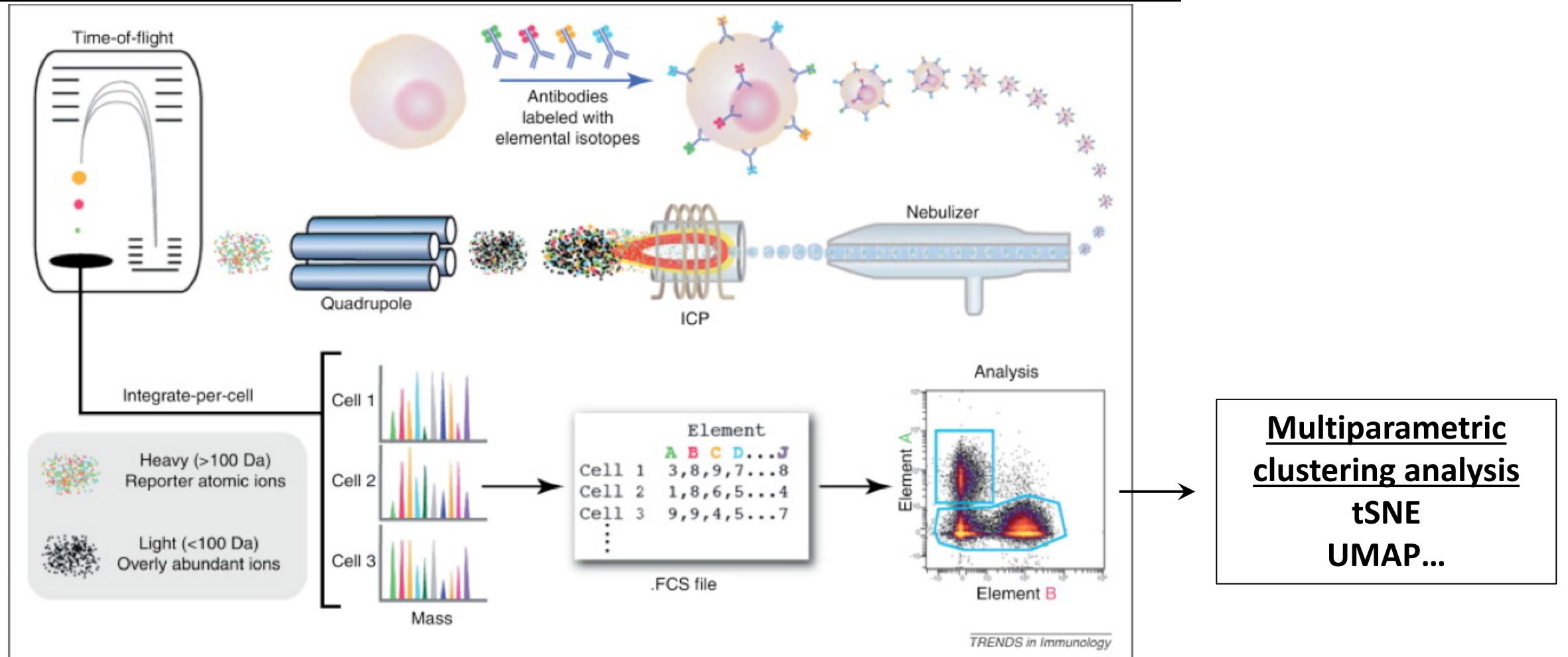


Shigella
infection

Surface marker		
viability	CD103	CD11c
CD45	CD25	CD66b
CD3	CD1c	CD27
CD4	CD11b	CD123
CD8	CD56	CD117
CD20	CD98	CD24
CD19	CD122	CD69
CD16	CD44	CD127
CD33	BDCA4	CCR6
HLA_DR	CXCR3	CD161
CRTh2	CCR7	CD45RA
CD14	PD1	CD45RO
CD94	CD38	CD62L



CyTOF technology



Adapted from Bendall et al, 2012, Trends Immunol.

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