

*Correlates of enteric vaccine-induced protection*  
*Annecy, 21-23 March 2016*

# **Th1, Th17 and T follicular helper cell responses to oral vaccination**

**Anna Lundgren**

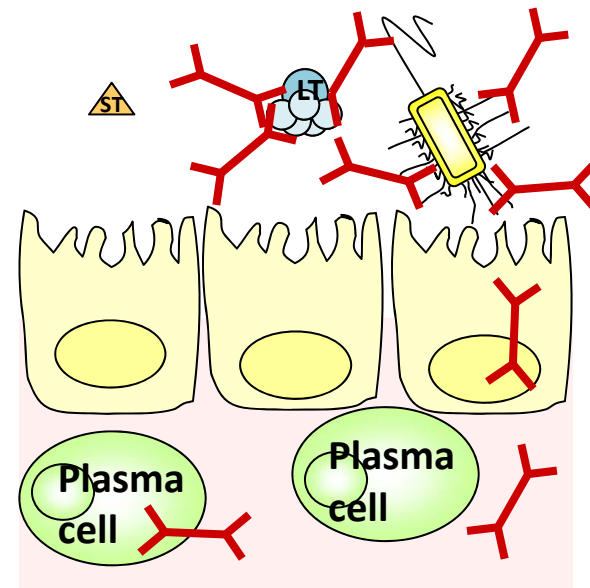
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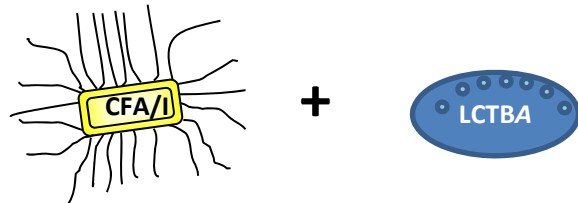
# Enterotoxigenic *E. coli* diarrhea

- Enterotoxigenic *E. coli* (ETEC) is an important cause of childhood as well as traveller's diarrhea.
- ETEC adheres to the intestinal epithelium via colonization factors (CFs) and produces LT and/or ST toxins, which cause diarrhea.
- Intestinal secretory IgA antibodies against ETEC CFs and LT cooperate synergistically for protection against ETEC.



# Clinical trials of a new oral inactivated ETEC vaccine

## Prototype ETEC vaccine

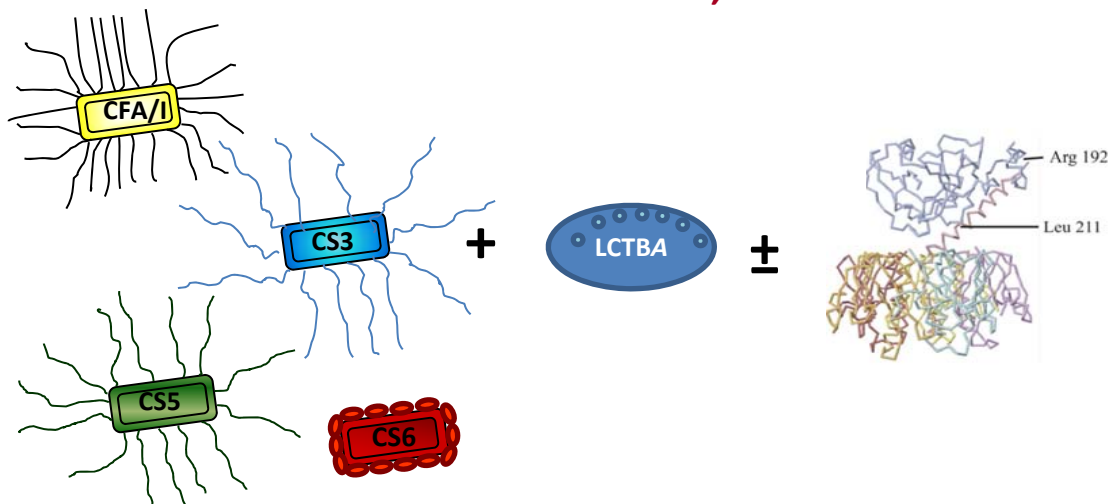


## Prototype vaccine trial

$n=59$

2 oral doses, two weeks apart

## Multivalent ETEC vaccine; ETVAX



Total:  $10^{11}$  bacteria

LTB/CTB subunit

dmLT adjuvant

*>4 fold higher expression of CFs than in previous generations of ETEC vaccine; CS6 added*

## Multivalent vaccine (ETVAX) trial

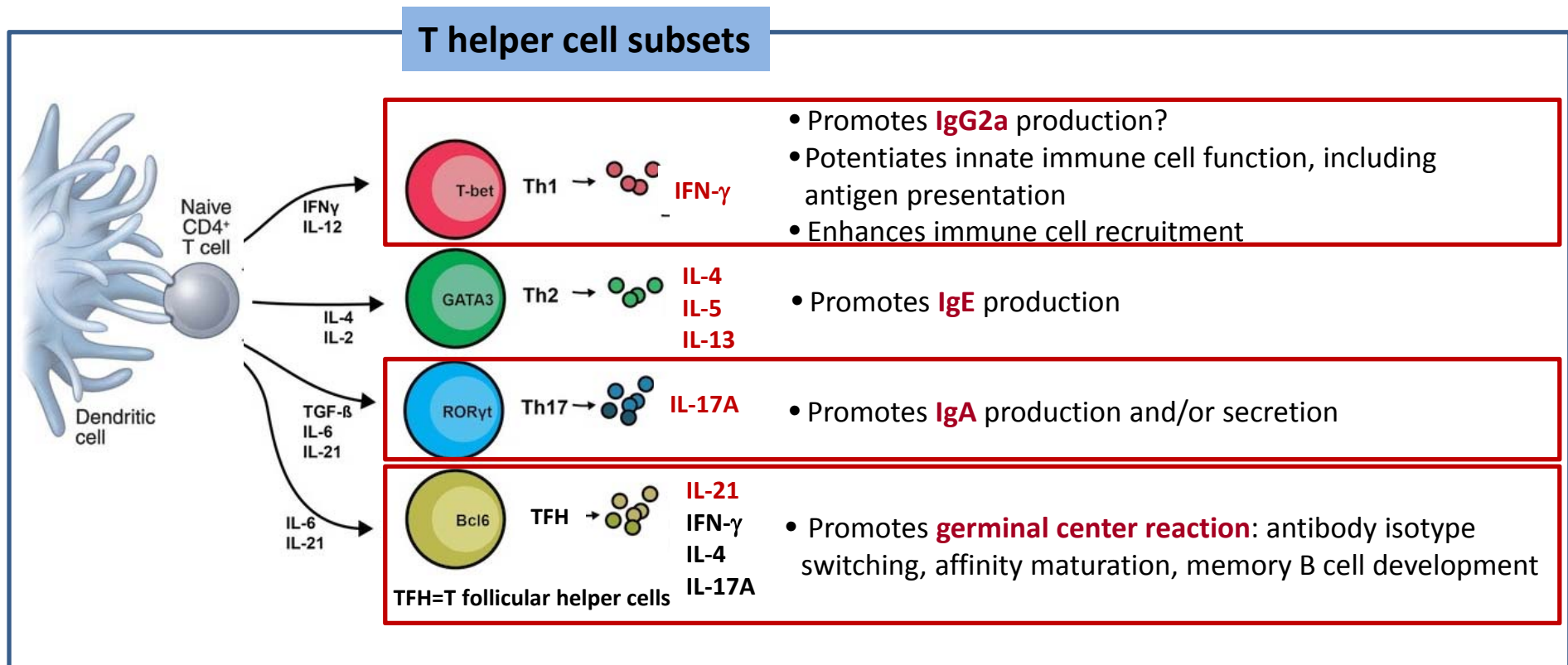
$n=129$

2 oral doses of ETVAX  $\pm$  dmLT, two weeks apart

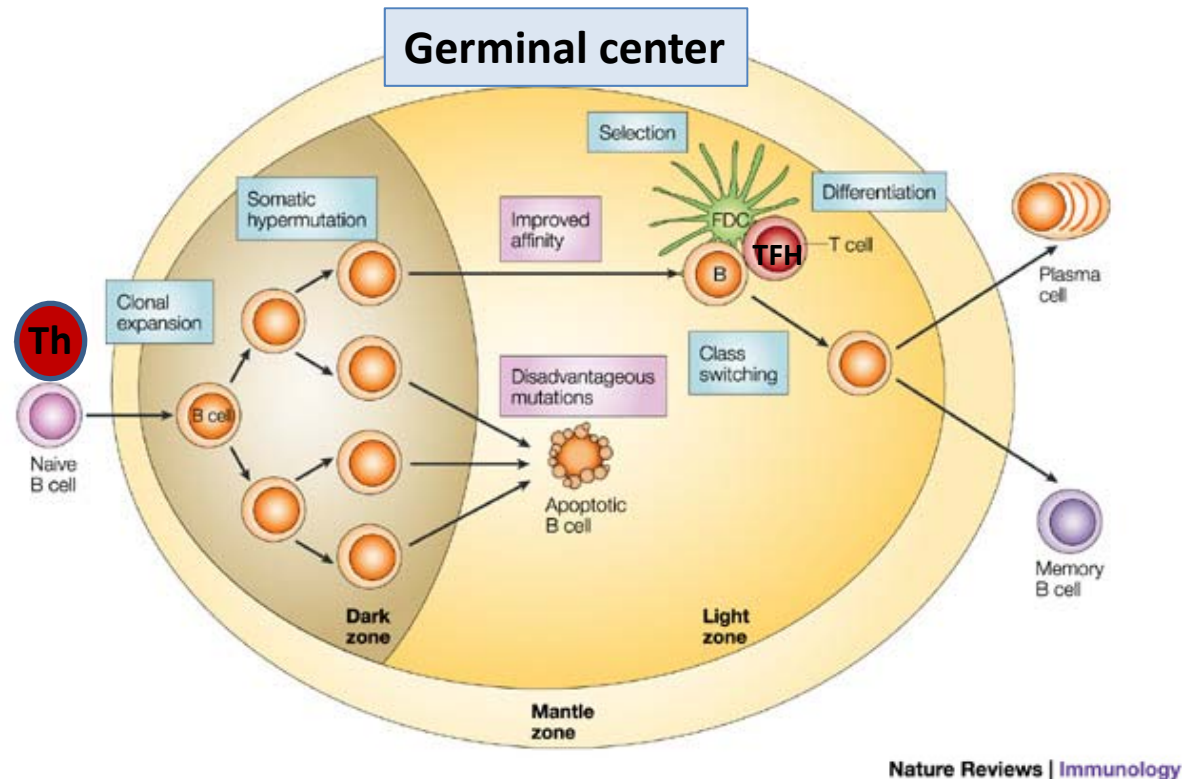
- The prototype and the multivalent ETEC vaccines were highly immunogenic and induced circulating antibody secreting cell and fecal antibody responses to all vaccine CFs and LTB. (Lundgren A. & Svennerholm AM et al., Vaccine 2013 & 2014)

# Influence of T cells on antibody production

- T helper cells can regulate B cell responses both directly and indirectly.



# T follicular helper cells promote B cell responses

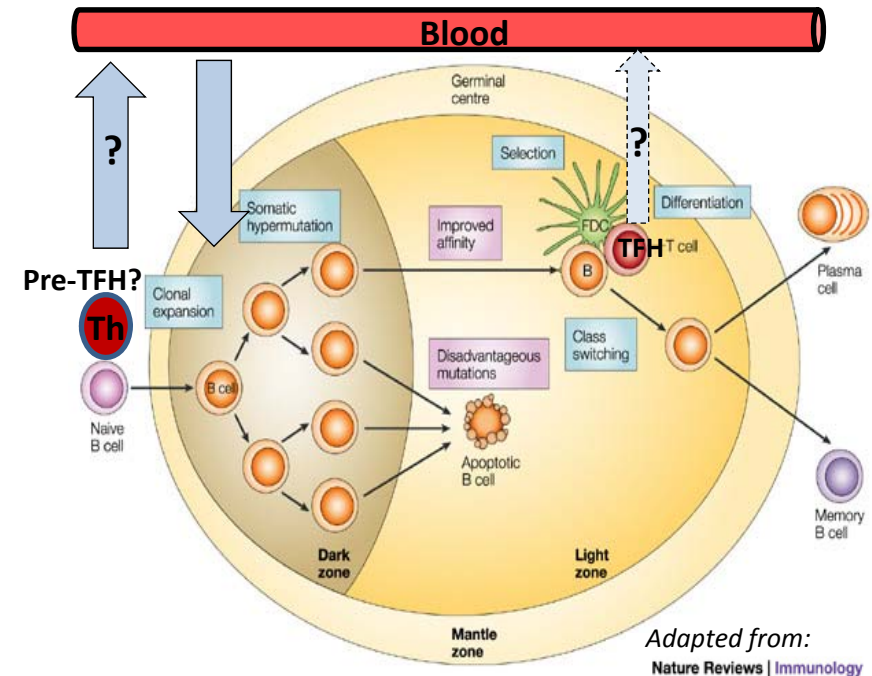
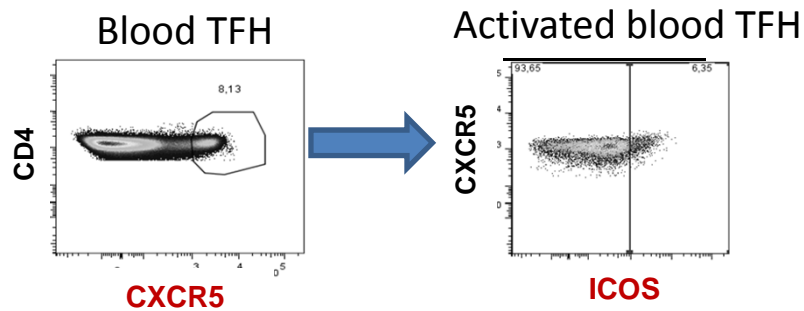


## T follicular helper (TFH) cells promote:

- antibody affinity maturation
- antibody isotype switching
- memory B cell generation
- long lived plasma cell generation

# Blood TFH-like cells

- T cells with TFH-like phenotype (**CD4+CXCR5+**) and **function** are present in human peripheral blood. (Morita R. et al., *Immunity* 2011, Locci M. et al., *Immunity* 2013)

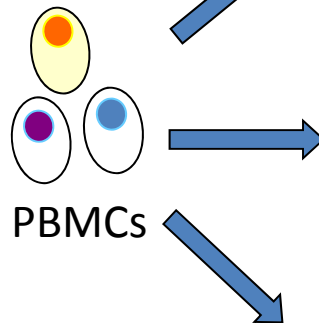
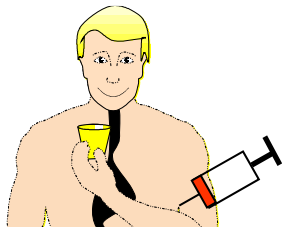


- Blood TFH-like cells promote B cell differentiation and antibody secretion via **IL-21**. (Morita R. et al., *Immunity* 2011)
- Increased frequencies of activated TFH-like cells in blood may reflect **active germinal center reactions** and can be related to production of **functional antibodies** (parenteral influenza vaccination, HIV infection etc). (He. J. et al., *Immunity* 2013, Bentebibel, S-E et al., *Sci Transl Med* 2013, Locci M. et al., *Immunity* 2013)

# Aims and methods

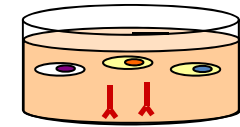
- Does oral ETEC vaccination induce T cell responses to CFs and LTB?
  - Which type of T cell responses?
  - Influence of dmLT?
  - Relation between T cell and B cell responses?
  - Can T cell response markers be used to predict B cell memory?

Swedish adults  
orally immunized with  
**ETEC-vaccine**



## Antibodies in lymphocyte supernatants assay (ALS)

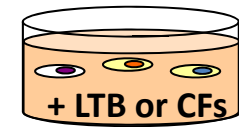
- Antibody secreting cells



Antibody ELISA

## T cell stimulation assay - cytokine analysis

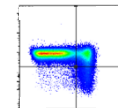
- **Th1:** IFN- $\gamma$
- **Th2:** IL-4, IL-5, IL-13
- **Th17:** IL-17A
- **Treg:** IL-10
- **TFH:** IL-21
- **Proinflammatory:** IL-6, IL-8, TNF



Cytokine ELISA/  
Chemiluminescence  
assay

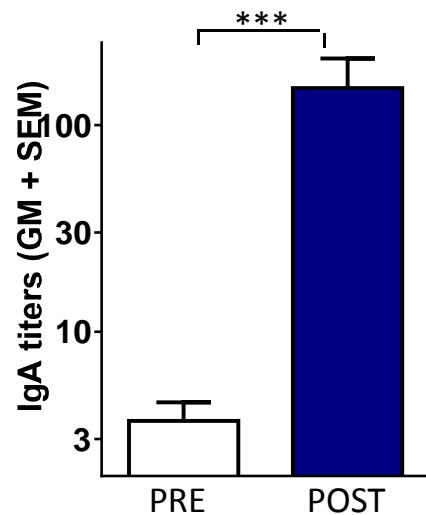
## Flow cytometry

- T follicular helper cells



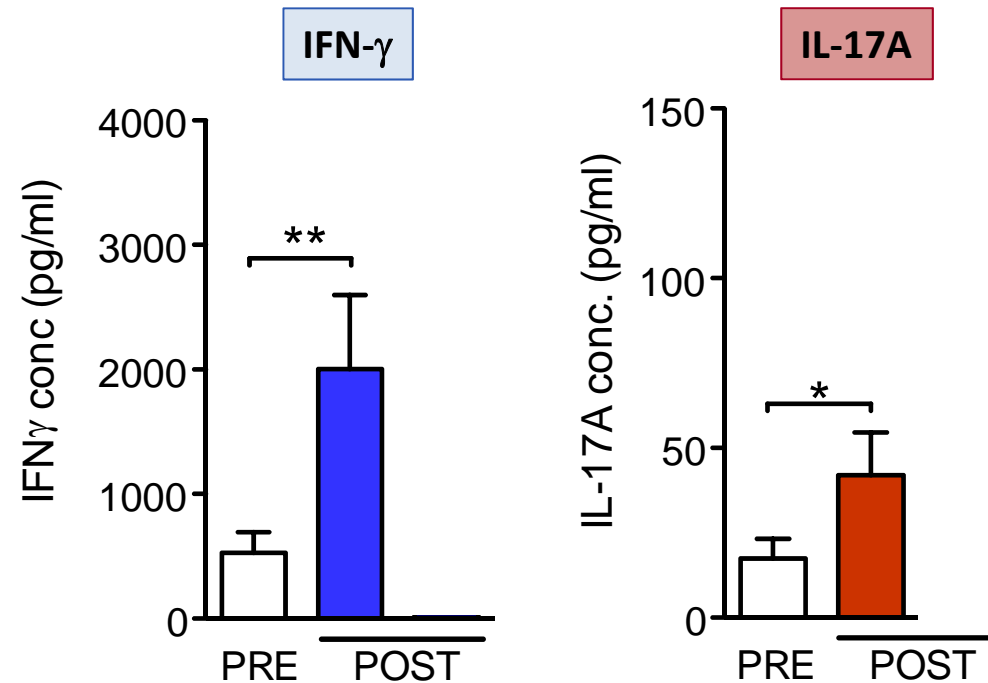
# B and T cell responses to the prototype ETEC vaccine

Anti-LTB **IgA ALS** response



Lundgren A. et al, Vaccine 2013

Anti-LTB **T cell** response



Leach S. et al., PlosONE, 2012

- The prototype ETEC vaccine induced T cell responses to LTB.
- dmLT enhanced IL-17A and IFN- $\gamma$  responses to LTB *in vitro*.



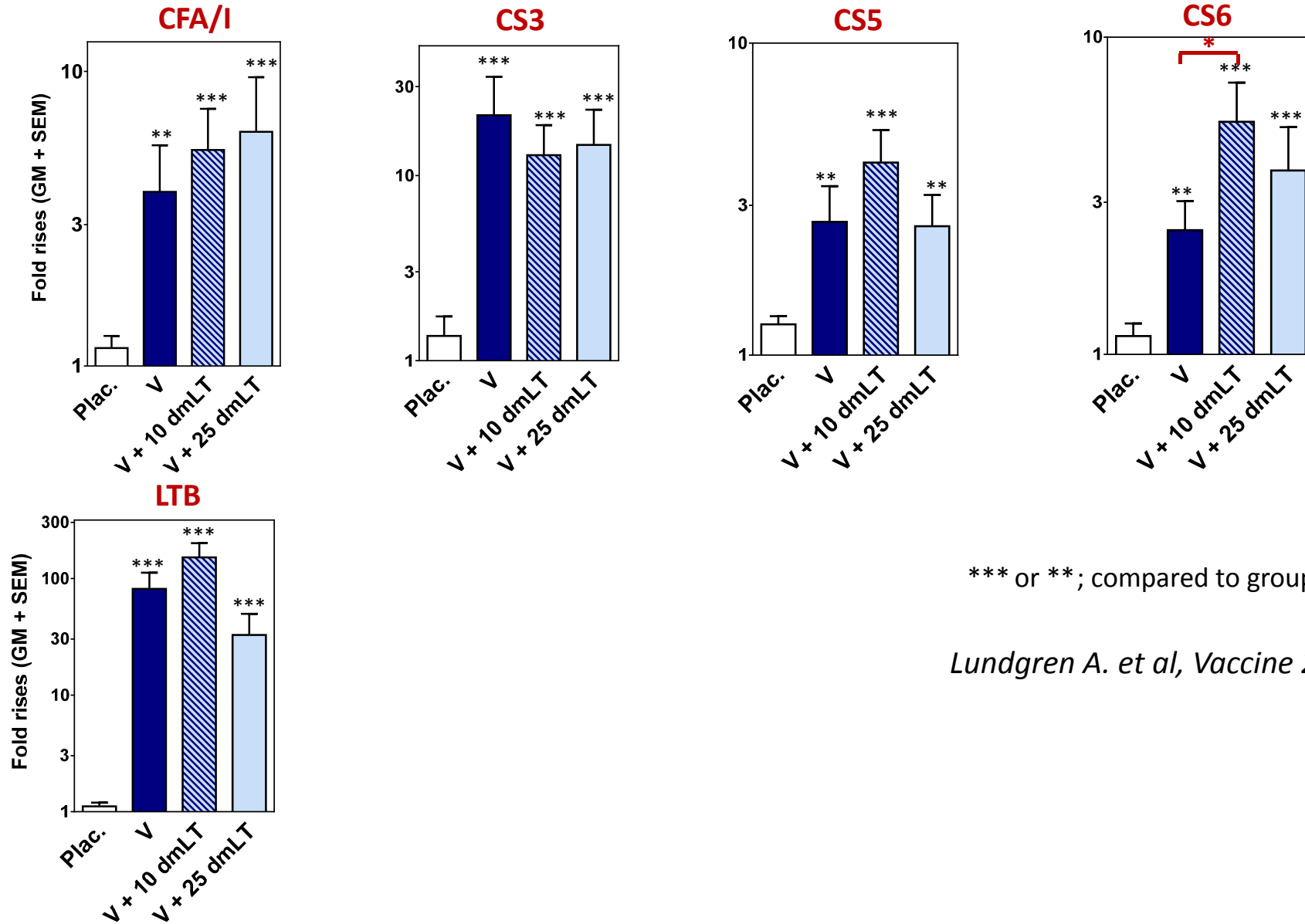
# Analysis of T cell responses to the multivalent ETEC vaccine (ETVAX)

## Study groups

		Total no of subjects	No of subjects in T cell studies
(A)	Placebo (vaccine buffer)	34	0
<b>B</b>	ETVAX	35	17
<b>C</b>	ETVAX + 10 $\mu$ g dmLT	30	17
<b>D</b>	ETVAX + 25 $\mu$ g dmLT	30	18
	<b>Total</b>	<b>129</b>	<b>52</b>

*Unpublished T cell response data has been removed from this online presentation.*

# ETVAX induced IgA ALS responses to both CFs and LTB



\*\*\* or \*\*; compared to group A

Lundgren A. et al, Vaccine 2014

➤ No clear adjuvant effect of dmLT, but a trend for adjuvant effect of low dose dmLT for most antigens; significant for CS6.

# Conclusions

- Oral ETVAX immunization induced significant Th1 and Th17 responses to all vaccine CFs and LTB.
- Strongest T cell responses to CS6 were induced by ETVAX + 10  $\mu$ g dmLT, consistent with enhanced mucosal IgA responses against CS6 in this group.
- ETVAX also induced a blood TFH response.
- The gut homing phenotype of activated blood TFH cells and the correlation with vaccine specific ALS responses suggest that blood TFH cells may reflect ongoing mucosal germinal center responses.
- TFH responses in blood may be used as a surrogate for mucosal memory B cell development.

# Acknowledgements

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