Food Antigen Transport Across the Intestinal Epithelium: Outcomes and Consequences

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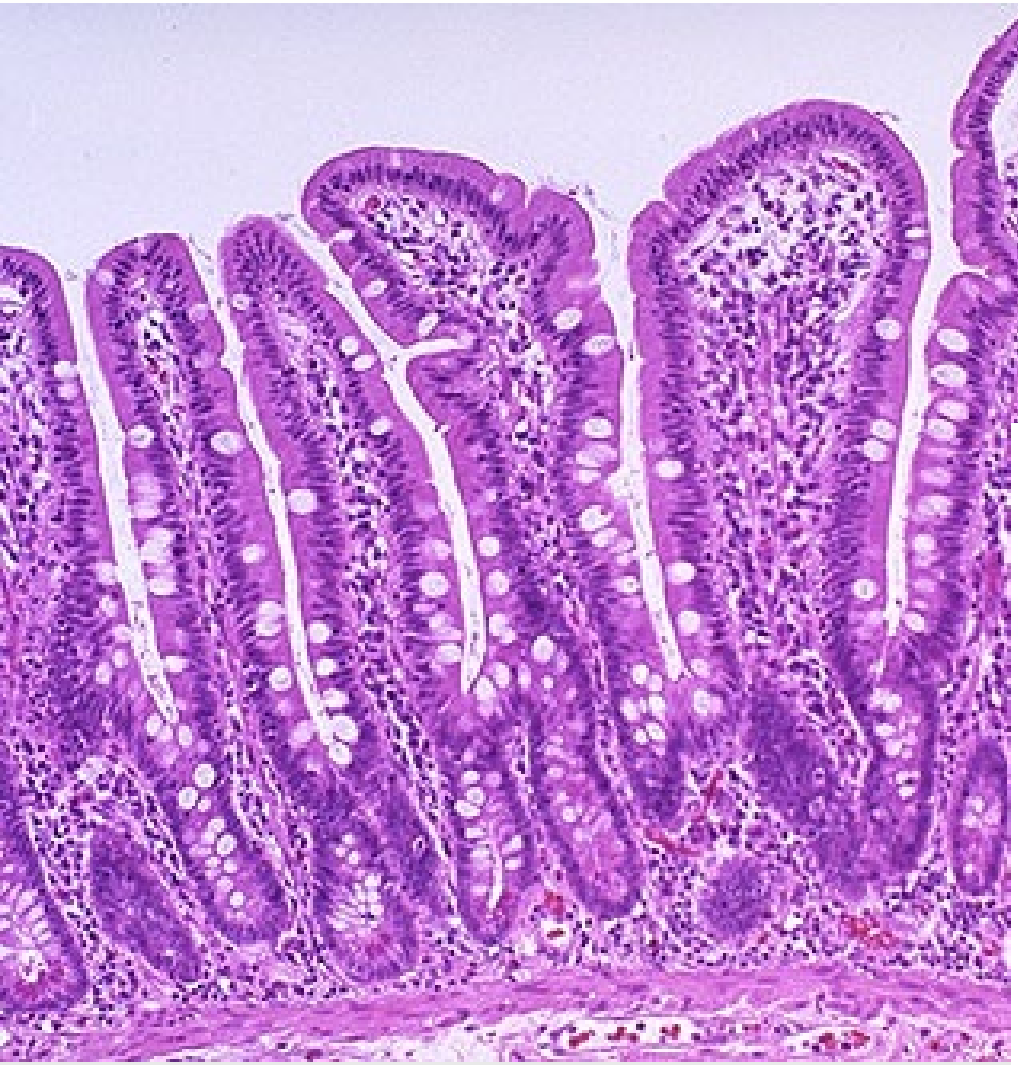
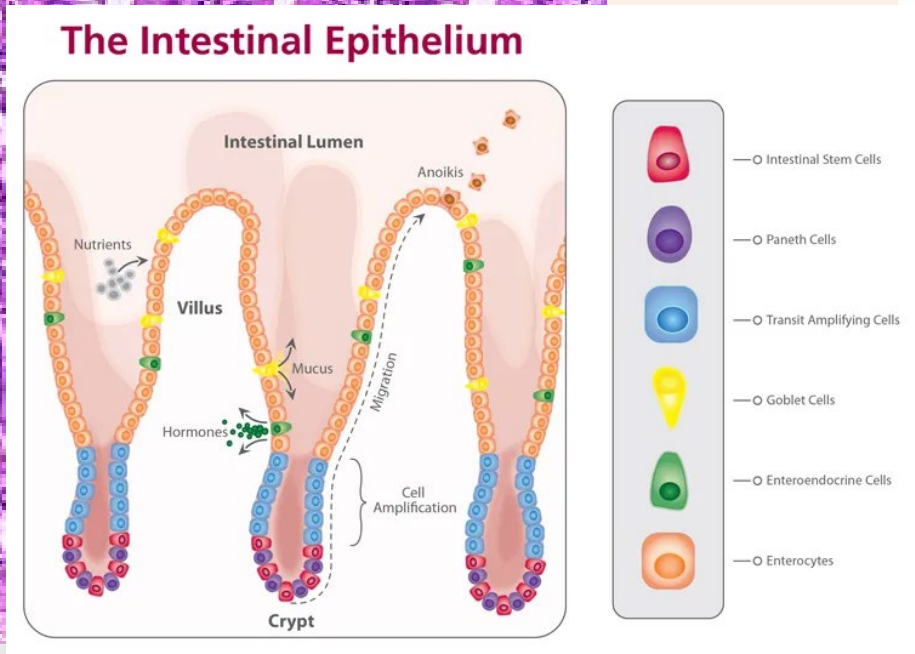
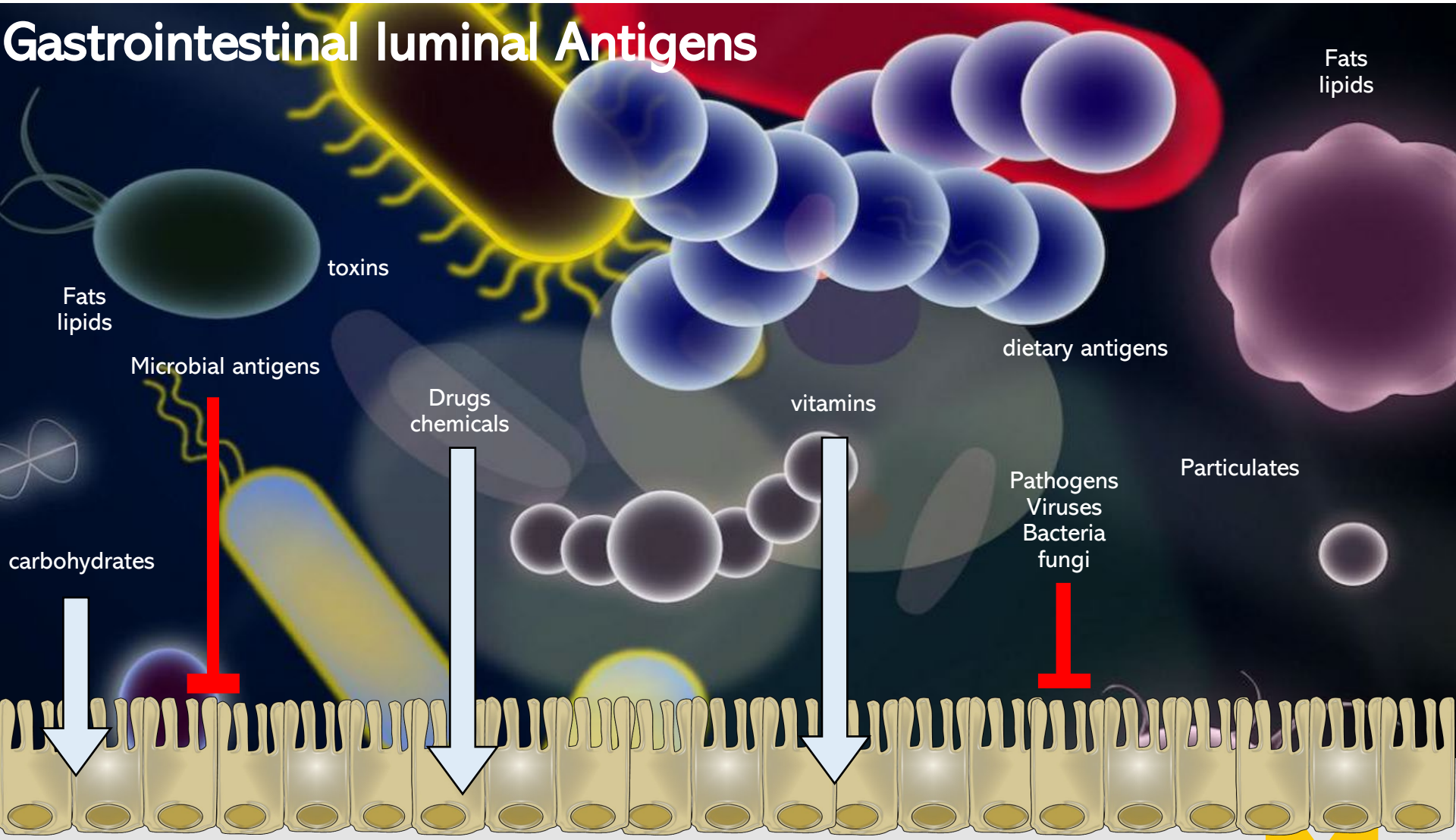
Disclosures

- Grant/Research Support: Regeneron Pharmaceuticals

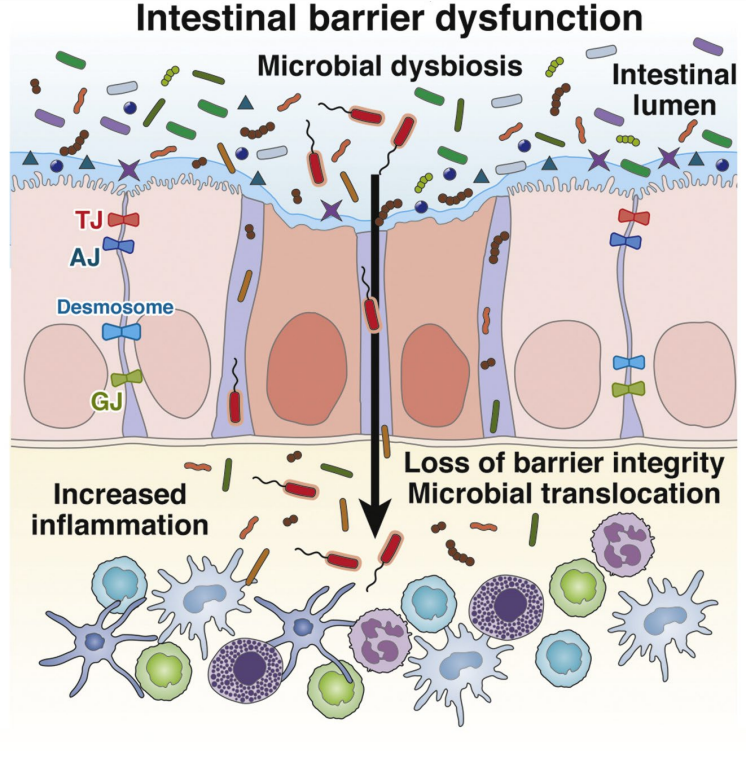
Anatomy of the Gastrointestinal Tract (small intestine)






Gastrointestinal luminal Antigens
Fats
lipids
toxins
Fats
lipids
Microbial antigens
dietary antigens
Drugs
chemicals
vitamins
Particulates
Pathogens
Viruses
Bacteria
fungi
carbohydrates

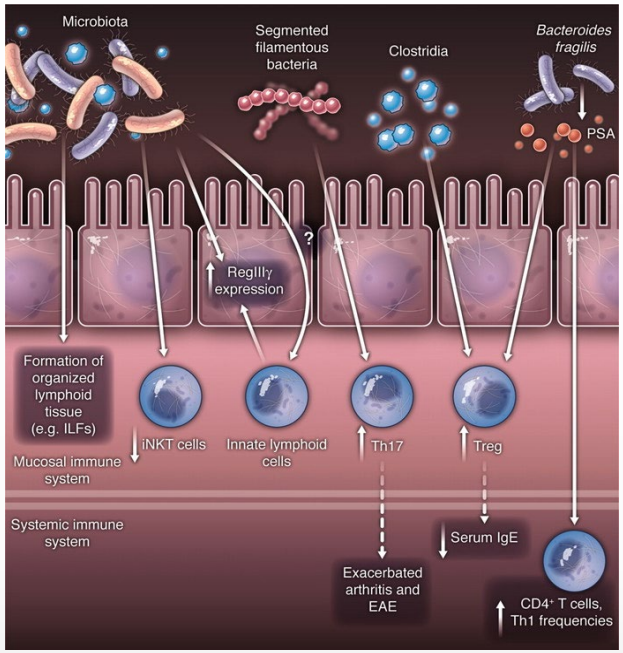
Intestinal epithelial barrier Dysfunction



Food Hypersensitivity
IBD
Autoimmunity
Metabolic Disorders

Gastrointestinal antigens and metabolites passage across the intestinal epithelium and engage with immune compartment.

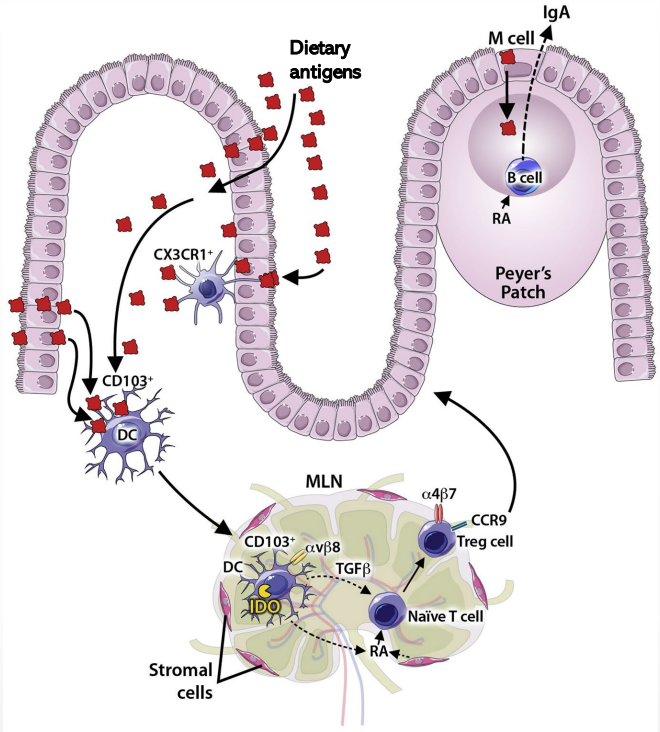
Gastrointestinal antigens and metabolites



Immune education

CD4⁺ T cell repertoire
Innate Lymphoid Cell (ILCs)

Dietary antigens



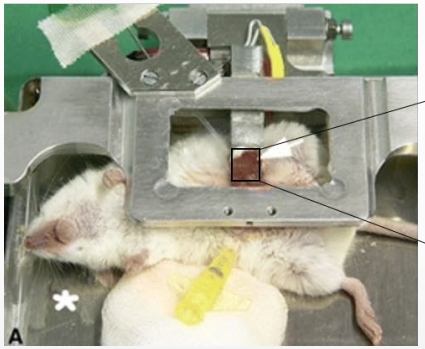
Oral Tolerance

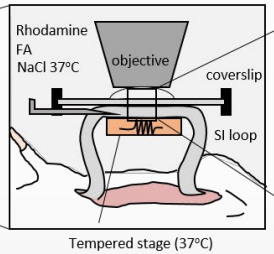
Interactions Between the Microbiota and the Immune System, 2012 Science 336 1268-1273.
Molecular and Cellular Mechanisms of Food Allergy and Tolerance 2016 JACI 137 984-997.

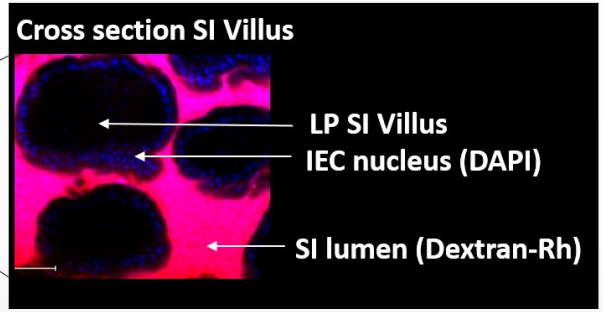
Mechanisms of gastrointestinal antigens (dietary antigens) passage across the intestinal epithelium are largely unclear?

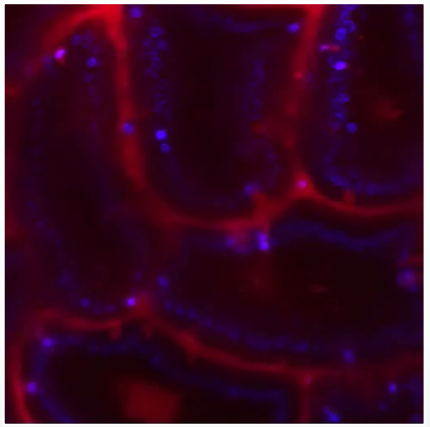
How do food allergens translocate across the SI intestinal epithelium

Intra luminal injection
Clinically relevant FA
(PN, SM or Egg)- Alexa^{647}
0 – 30 minutes following injection





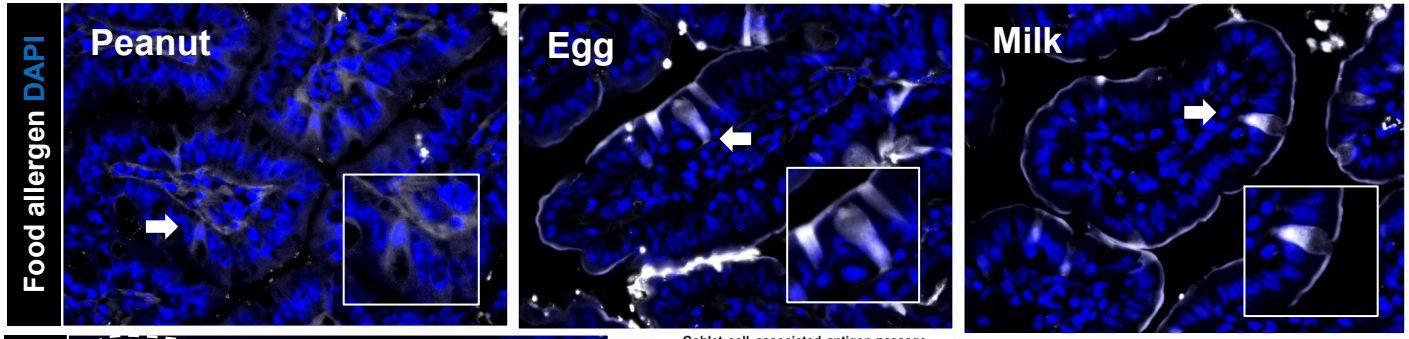


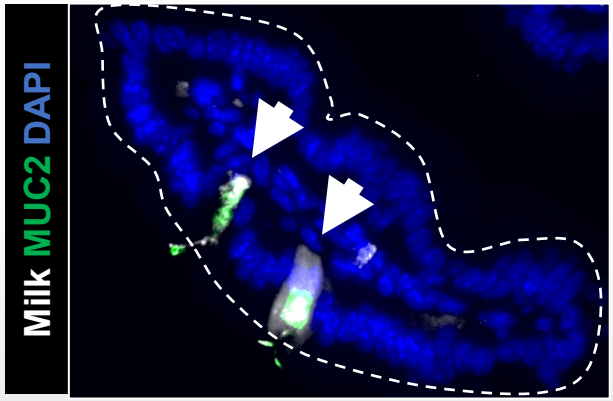


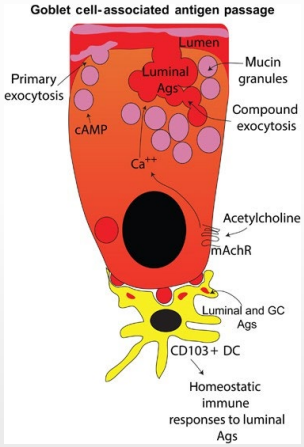
***In vivo*- Mouse SI 2P Imaging**

Noah et al., JACI 2020

Food Allergens passage across the intestinal epithelial layer via Goblet cell antigen passages







Goblet cell antigen passages (GAPs)
Steady state
Restricted to GCs- villus
M4AchR-dependent

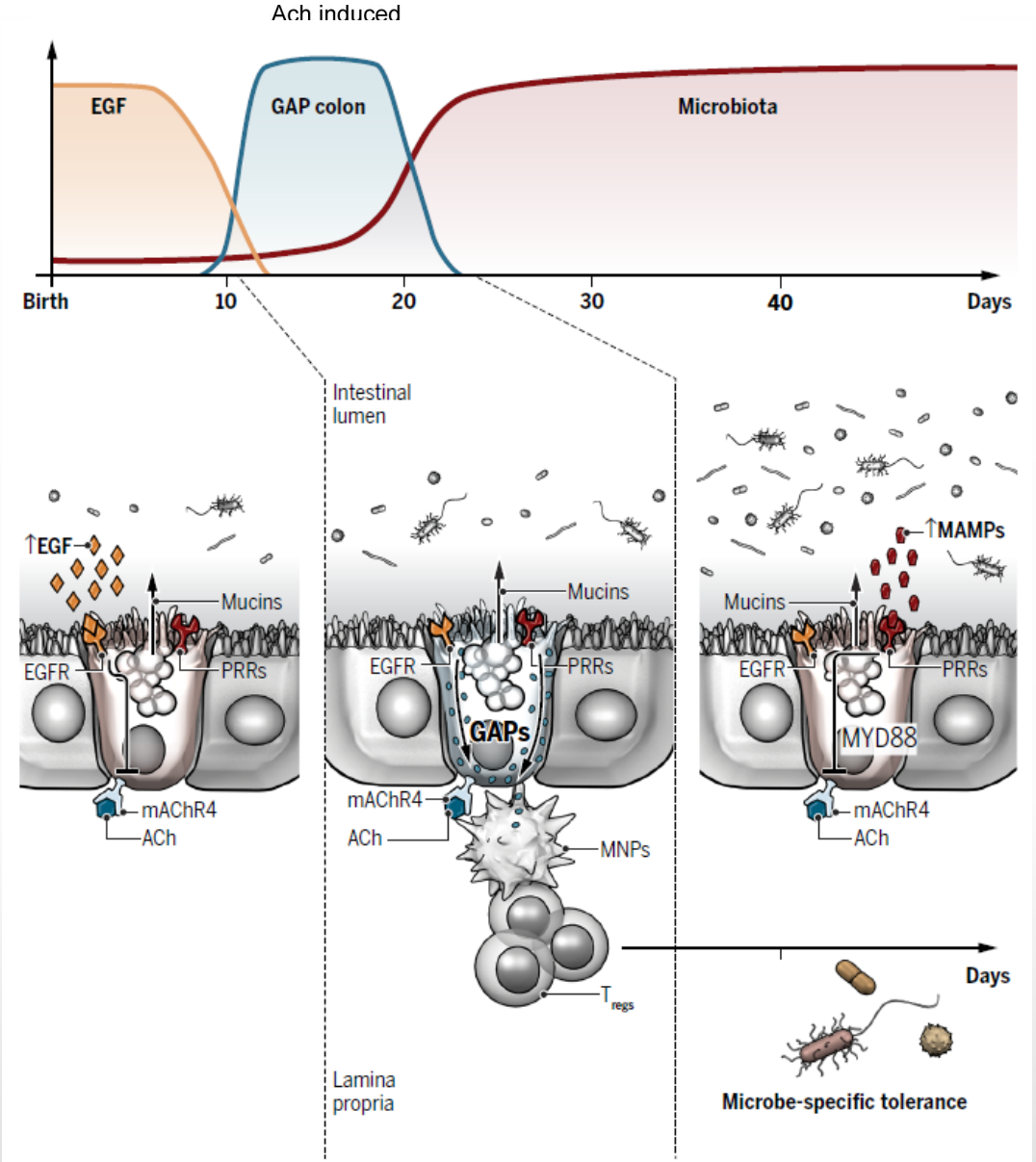
< 110 kDa
innate antigens
Commensal antigens

Passage antigen to Dendritic Cells

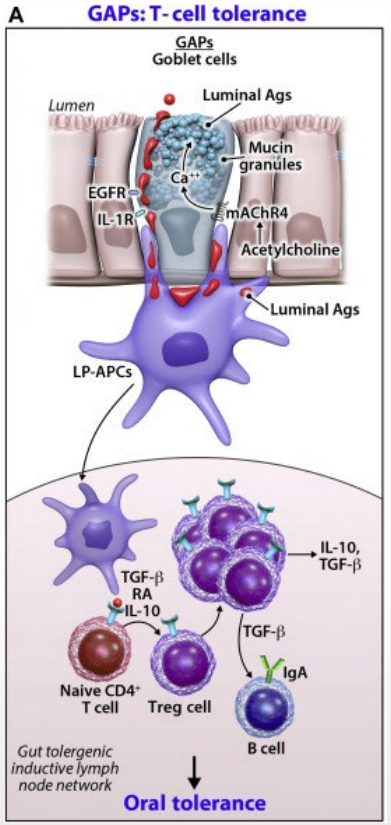
Miller, Knoops, Newberry, 2014 Mucosal Immunology
Newberry and Hogan, JACI, 2021

Conclusions: Goblet cell Antigen Passages (GAPs)

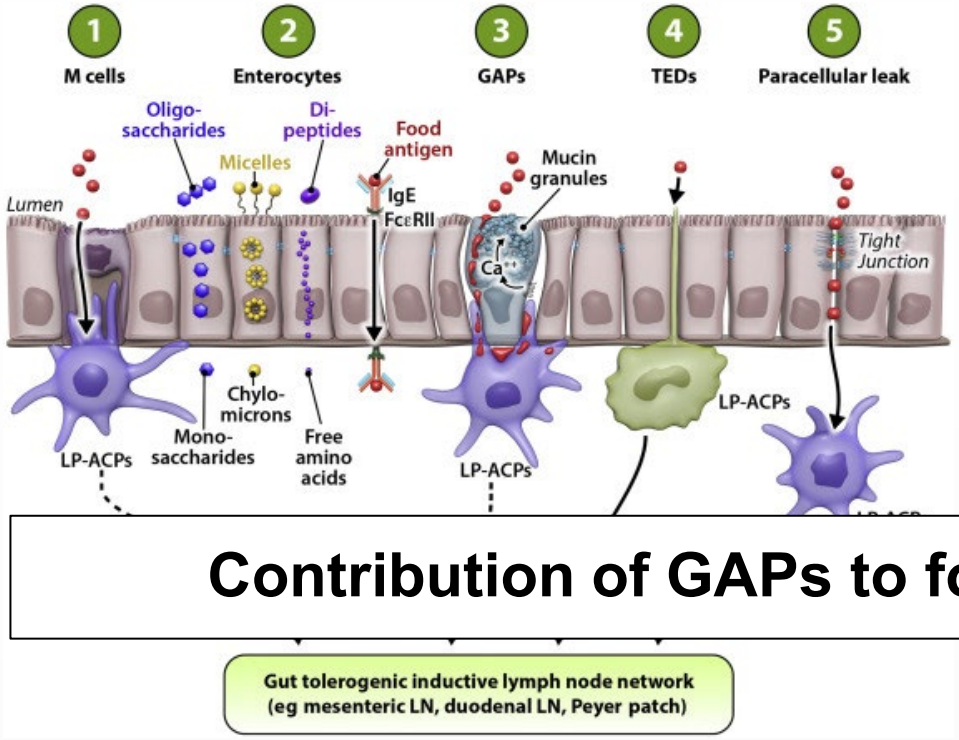
Gustafsson et al., Elife, 2021
Knoops et al., Front Immunol 2021
Knoops et al., JCI Insight 2020
Kulkarni et al., Mucosal Immunol 2020
Noah et al., Frontiers Immunol 2021
Al Nabhani and Eberl, Sci Immunol 2017



Steady State



Mechanisms of Dietary antigen sampling by Immune Compartment?



Food allergen	Allergen name*	Mass (kDa)**
Chicken egg	*Gal d*1 (ovalbumin)	45
	*Gal d*2 (ovomucoid)	28
Cow's milk	α_{S1}-casein	23
	β-lactoglobulin	18.4
Soybean	*Gly m*1 A, *Gly m*1 B	8
	*Gly m*Bd (7S globulin)	30
Peanut	*Ara h*1	63.5
	*Ara h*2	17.5
Brazil nut	*Ber e*1 (2S albumin)	16–16.4
Yellow mustard	*Sin a*1 (2S albumin)	15
Oriental mustard	*Bra j*1 (2S albumin)	16
Codfish	*Gad c*1	13
Shrimp	*Pan a*1 (tropomyosin)	36

*According to the recommendations of the IUIS Subcommittee for Allergen Nomenclature. **Molecular mass of the allergen.

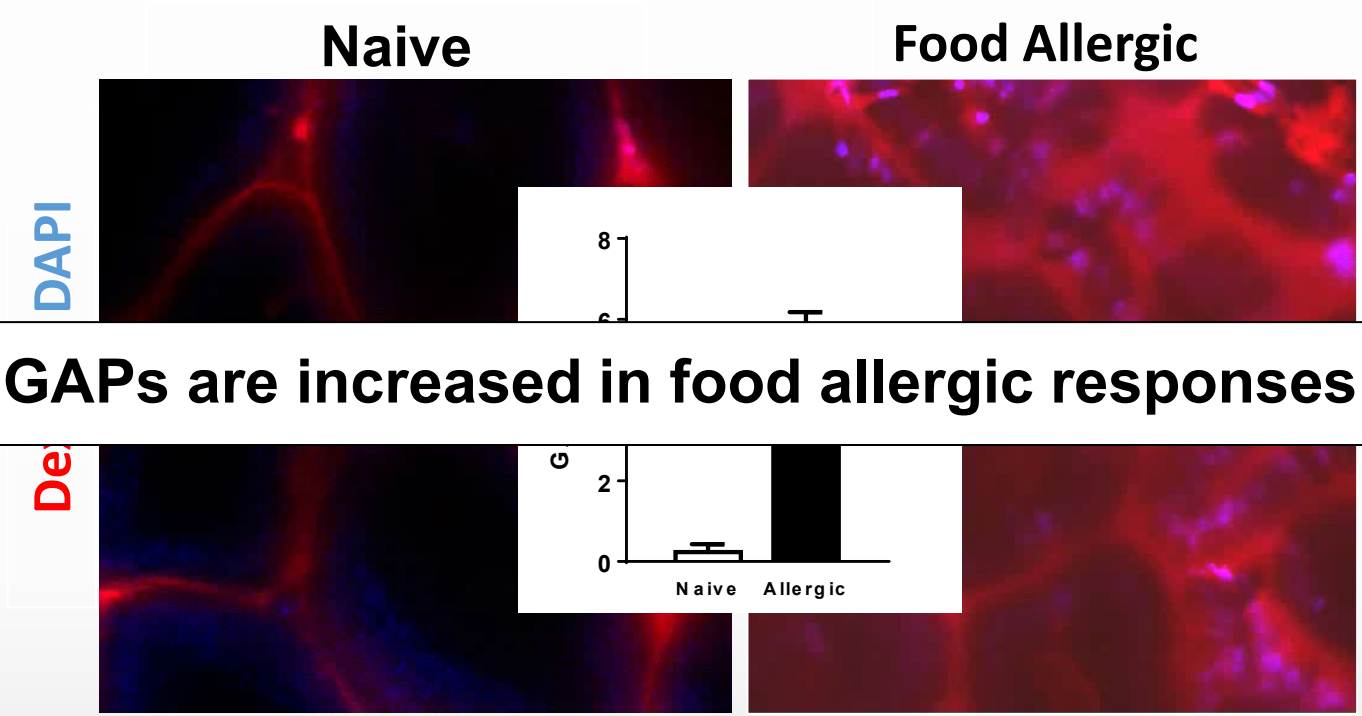
Contribution of GAPs to food allergic responses?

	1	3	4	5	
	Soluble proteins, Aggregated proteins sIgA immune complex	Soluble proteins and peptides < 110kDa	Bacteria and debris	Small peptides and molecules < 600 Da	**Types Antigens**
	FAE and PPs	SI and distal colon	Terminal ileum	Whole GI tract	**Location**
	~10% FAE	3-5 GAPs / villus	1 TED / 100 Villi	-	**Frequency**

Induce oral tolerance
Sustain oral tolerance
Immune education

Newberry and Hogan, JACI, 2021
Kaminogawa et al., Allergology Intern, 1999

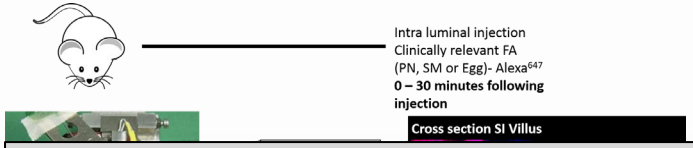
Dietary antigen translocate across the SI intestinal epithelium in food allergic mice

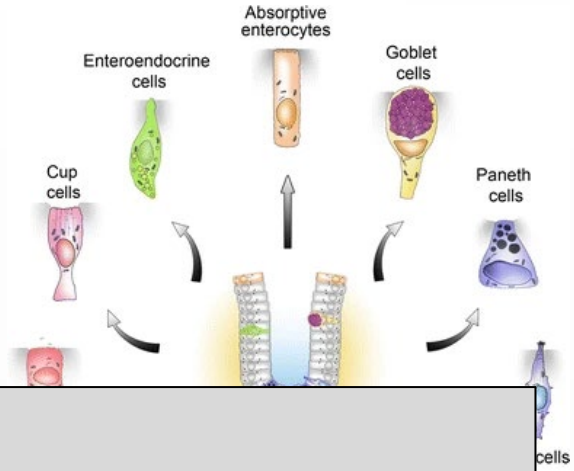


GAPs are increased in food allergic responses

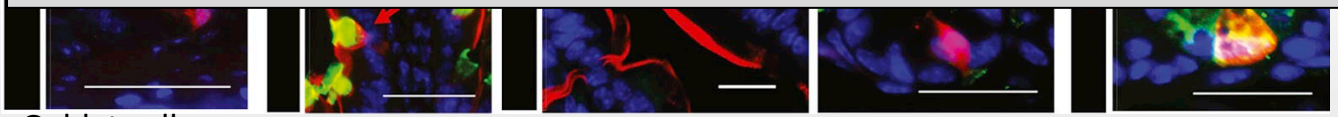
↑ Antigen passage frequency in SI of food allergic Mice

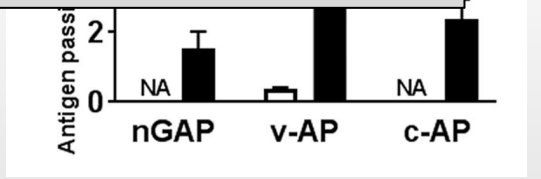
Food allergens translocate across the SI intestinal epithelium via a non-canonical antigen passages in Food Allergic Mice





Under food allergic conditions antigen passage patterning and landscape is dysregulated
Multiple intestinal epithelial cells of the secretory lineage- Take up food antigens
SAPs – secretory cell antigen passages
Antigen passages can be observed in the villus and crypt epithelium



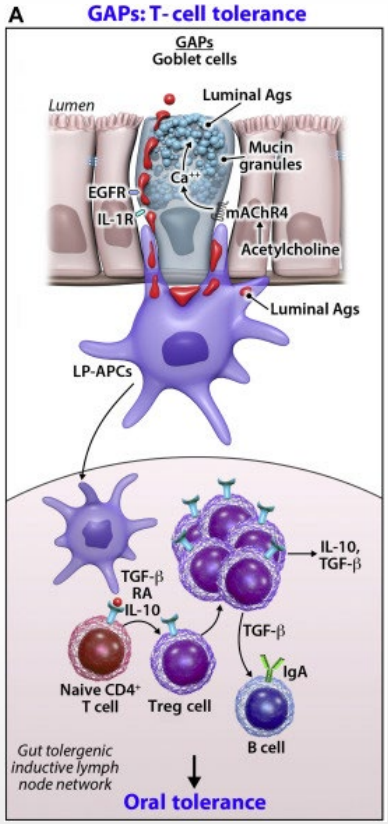


Goblet cells
ChrA- Endoendocrine cells- Villus
Paneth cells- Crypt

Noah et al., JACI 2020

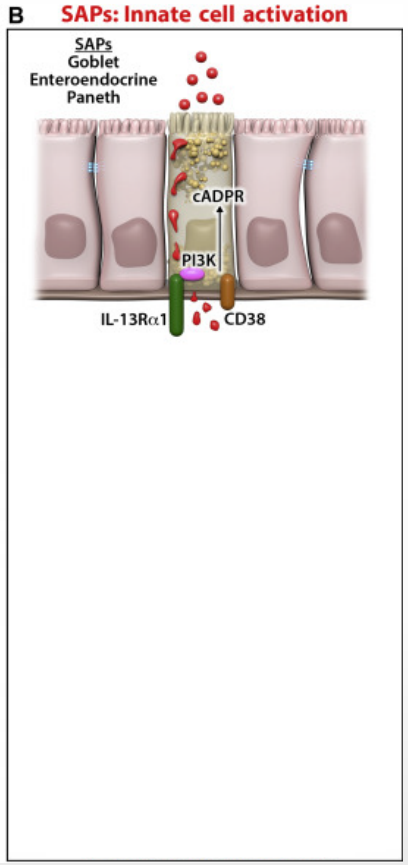
Intestinal epithelial Cell Antigen passages:

Steady State
Goblet cell restricted



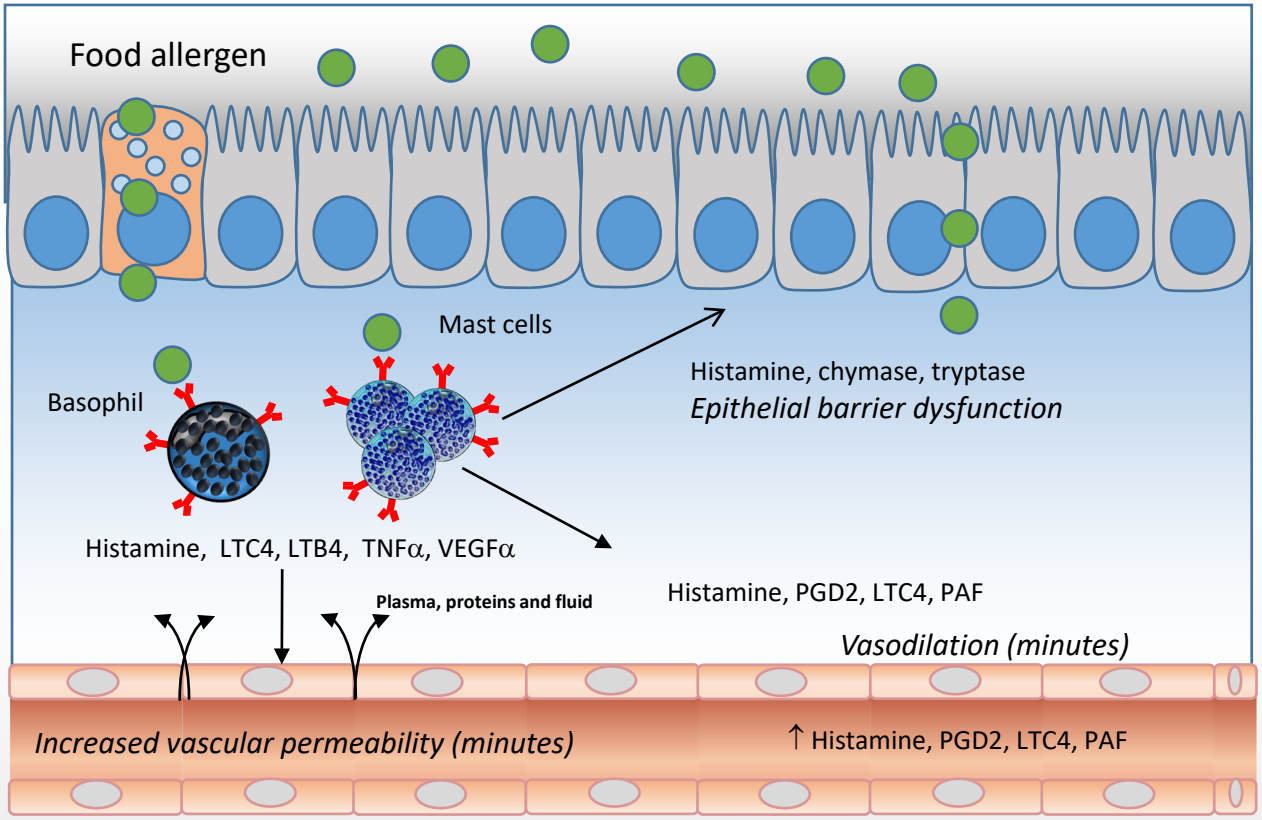
Gustafsson et al., Elife, 2021
Knoops et al., Front Immunol 2021
Knoops et al., JCI Insight 2020
Kulkarni et al., Mucosal Immunol 2020
Noah et al., Frontiers Immunol 2021

Food Allergic State
All secretory epithelial cells



Noah et al., JACI 2019
Noah et al., Frontiers Immunol 2021

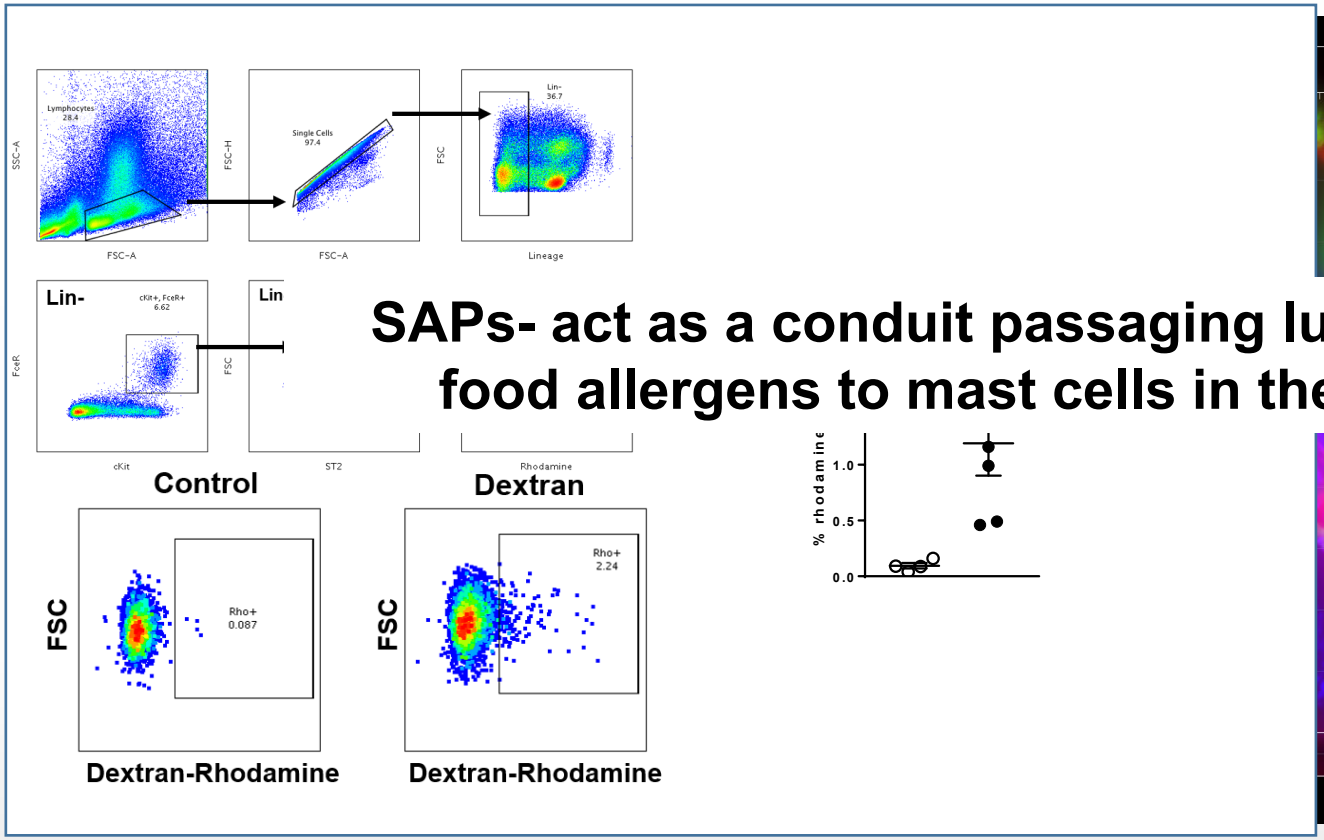
Do Secretory antigen passages induce Food allergic reaction?



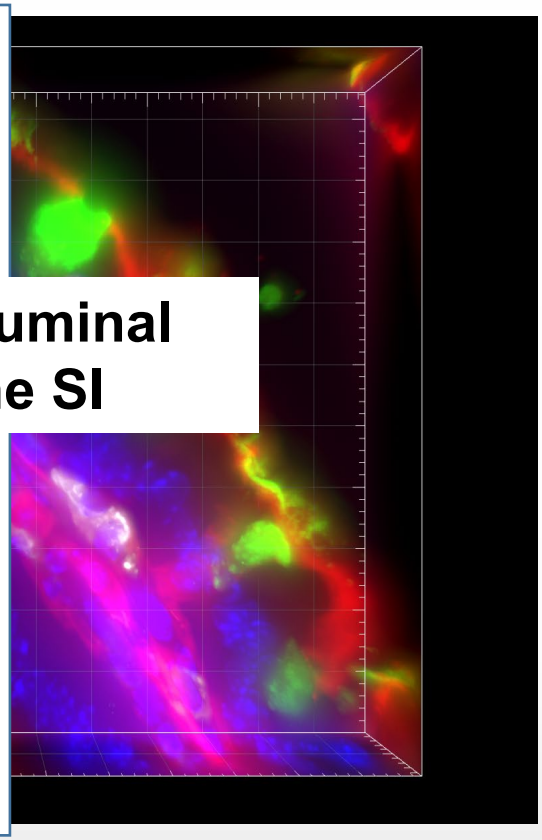
Do food allergens passage across the SI intestinal epithelium and stimulate an IgE-Mast cell response

Osterfeld et al., JACI 2010 125:469
Sledd et al., 2015 Immun Inflamm Dis. 2015 Sep 17;3(4):420-30
Yamani et al., JACI 2018 and Mucosal Immunology 2020

Secretory Antigen Passages- SAP

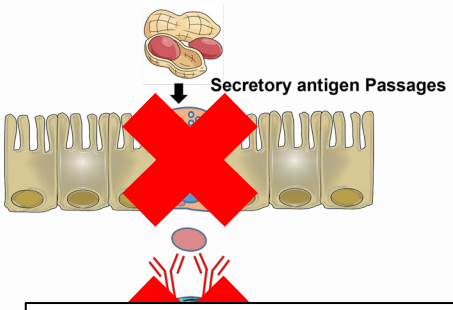


SAPs- act as a conduit passaging luminal food allergens to mast cells in the SI



Are SAPs required for a Food Allergic reaction?

Food reaction

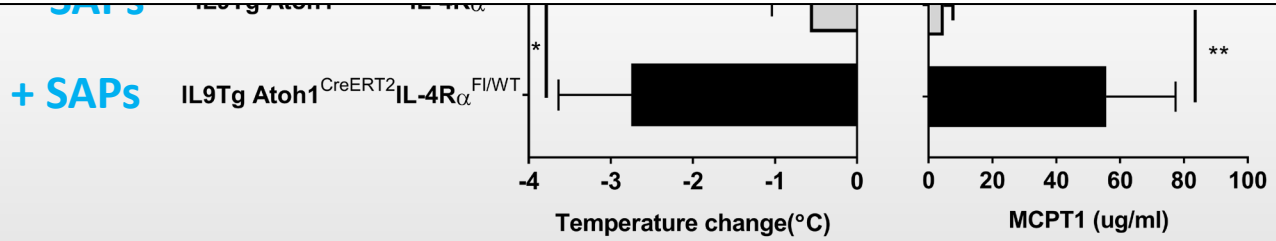


IL-9Tg Atoh^{CreERT2} IL-4Rα$^{Fl/Fl}$ mice
(3 x Tamoxifen / every other day)
Intestinal mast cells (IL-9)
± IL-4Rα secretory epithelial cells

o.g. TNP-BSA

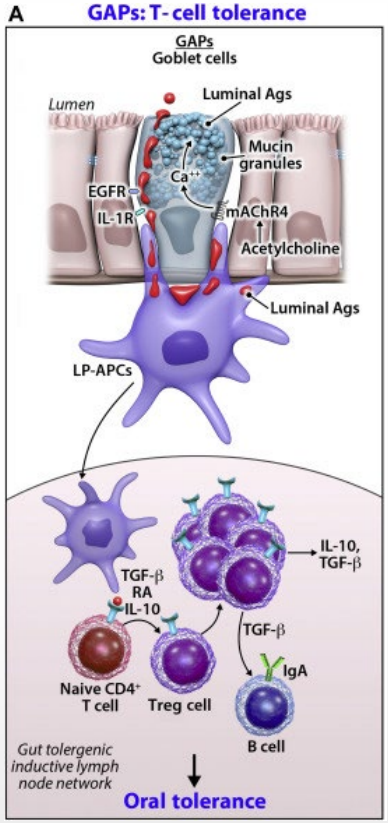
i.v.

IntSAPs- act as conduit luminal food allergens
Inhibition of SAPs prevent IgE-mast cell mediated food-induced reaction



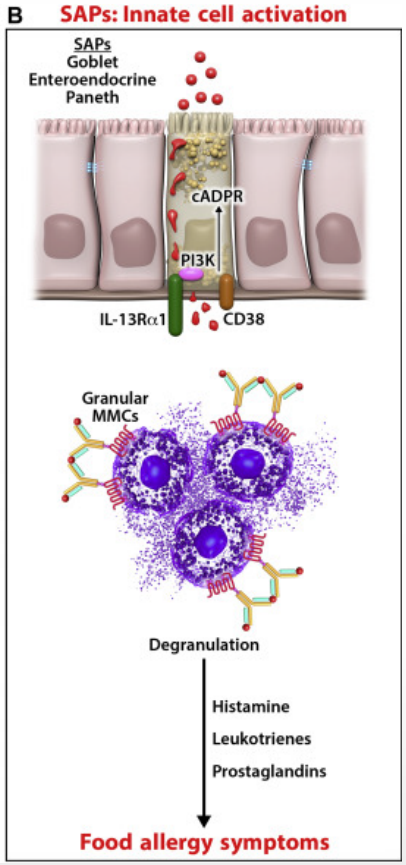
Intestinal epithelial Cell Antigen passages:

Steady State



Gustafsson et al., Elife, 2021
Knoops et al., Front Immunol 2021
Knoops et al., JCI Insight 2020
Kulkarni et al., Mucosal Immunol 2020
Noah et al., Frontiers Immunol 2021

Food Allergic State



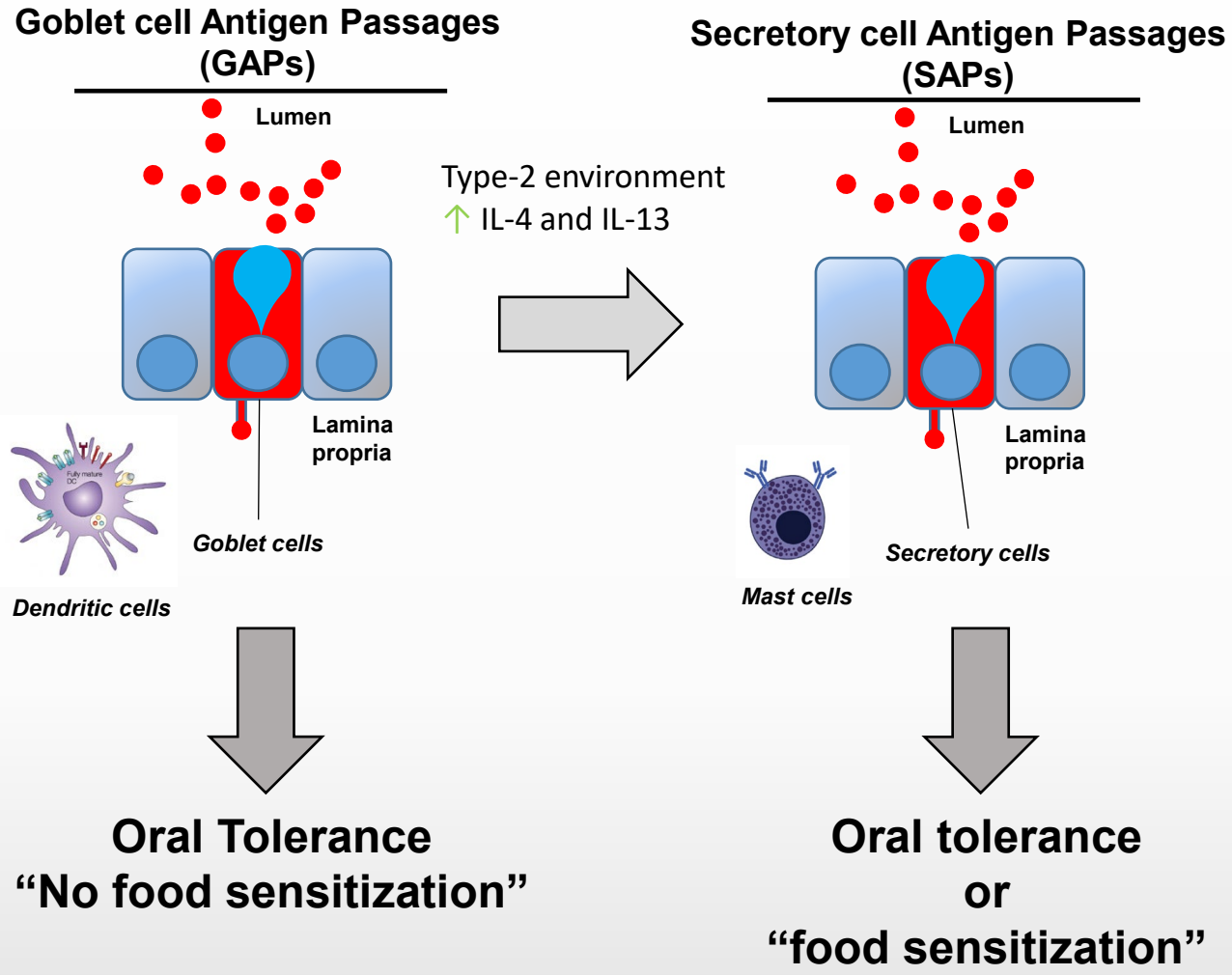
IntSAPs- conduit luminal food allergens

IntSAPs- Conserved in human tissue

Regulated by IL-13-IL-4Rα and CD38

Inhibition of SAPs prevent IgE-mast cell mediated food-induced reaction

Do alteration in Antigen passage patterning and landscape drive food sensitization?



Genetics of Food Allergy

Mechanisms of allergic diseases

Current insights into the genetics of food allergy

Kanika Kanchan, PhD,[a] Selene Clay, BA,[b] Haritz Irizar, PhD,[c,d] Supinda Bunyavanich, MD, MPH,[d,e] and Rasika A. Mathias, ScD[a] *Baltimore, Md; Chicago, Ill; London, United Kingdom; and New York, NY*

TABLE I. Genetic loci and genes associated with FA identified by GWASs, CGASs, and G × E interaction studies organized by functional categories

Functional categories	GWAS	CGAS	G × E
Skin barrier integrity	**FLG-AS1**,[7] **SERPINB7**|**SERPINB2**[7]	**FLG**,[8-12] **SPINK**[13,14]	**FLG**[15]
Vascular and endothelial cell factors	**ANGPT4**,[16] **CHCHD3**|**EXOC4**,[16] **CTNNA3**,[16] **SKAP1**[16]		
Innate immunity	**HLA-DPB1**,[17,18] **HLA-DRB1**,[17-19] IL26,[17] MALT1[20]	**CD14**,[21,22] HLA-B,[23] **HLA-DPB1**,[24] **HLA-DRB1**,[23-25] **IL10**,[26,27] **IL2**|**IL21***|**KIAA1109**,[12,28] TLR10/1/6*|FAM114A1,[28] TSLP*|WDR36[12]	MALT1[20]
Adaptive immunity	**C11orf30**|**LRRC32***|**LOC101928813**,[7,16] **HLA-DPB1**,[17,18] **HLA-DQA1**,[17-19] **HLA-DQA2**,[18,19] **HLA-DQB1**,[7,17-19,29] **HLA-DRA**,[18,19] **HLA-DRB1**,[17-19] HLA-DRB5,[18] IL26,[17] **IL4***|**KIF3A**,[7] MALT1[20]	**C11ORF30**|**LRRC32**,*[12,30] C-REL,[31] HLA-B,[23] **HLA-DPB1**,[24] **HLA-DQB1**,[23,24,32,33] **HLA-DRB1**,[23-25] **IL10**,[26,27] **IL2**|**IL21***|**KIAA1109**,[12,28] **KIF3A**|**IL13**,*[12,34] **STAT6**,[12,35] TSLP*|WDR36[12]	MALT1[20]
Immune modulation and regulation	IL26,[17] **IL4***|**KIF3A**,[7] **SERPINB7**|**SERPINB2**[7]	C-REL,[31] **IL10**,[26,27] IL28B,[36] **IL2**|**IL21***|**KIAA1109**,[12,28] **KIF3A**|**IL13**,*[12,34] **STAT6**[12,35]	DBP/GC[37]
Other	ADGB,[38] ARHGAP24,[16] ATP10A,[17] BCAS1,[17] **DLX2**|**ITGA6**,[16] FXR1,[17] GNPDA1|NDFIP1,[20] GYG1P2|RNU6-67P,[38] IER5L,[17] LINC00540|BASP1P1,[20] LINC00298|LINC00299,[20] LINC01260|KCNK15-AS1,[20] LINC01568|LOC101928035,[20] LINGO2,[17] LMX1A,[17] **LOC101927166**,[16] LOC101927947,[38] MDN1,[17] **MMP12**|**MMP13**,[16] NAV2,[17] PADI6,[20] PAFAH1B1,[17] PAX2,[17] PLAGL1,[17] PLPP7|PRRC2B,[20] PYROXD1,[17] RCC2|ARHGEF10L,[20] RGS21,[17] RIMS2,[17] RNF130,[17] SALL3,[17] SLC2A9,[17] SORBS2,[17] SV2C,[17] TES,[17] TRIM2,[20] **ZNF652**[38]	CCDC80,[12] CLEC16A|DEXI,[12] GLB1,[12] NAT2,[39] NLRP3,[40] OR10A3|NLRP10,[12] OVOL1,[12] TMEM232|SLC25A46,[12] TNFRSF6B|ZGPAT,[12] ZNF365,[12] **ZNF652**[12]	

Genes/loci that are bolded are those that are replicated within the study, or have evidence across multiple studies. For genetic loci mapping to multiple genes, the index gene for each category is marked with an asterisk (*).

Il4ra^{Y709F} mice -

Reflecting the enhanced IL-4 pathway phenotype in atopic patients

- Point mutation (Tyr – Phenylalanine substitution) in IL-4Rα
- Enhanced IL-4 signaling (similar phenotype to atopic humans)
- Can be orally sensitized to ovalbumin without adjuvant but with a minimum penetrance (< 20%)

Intestinal phenotypes

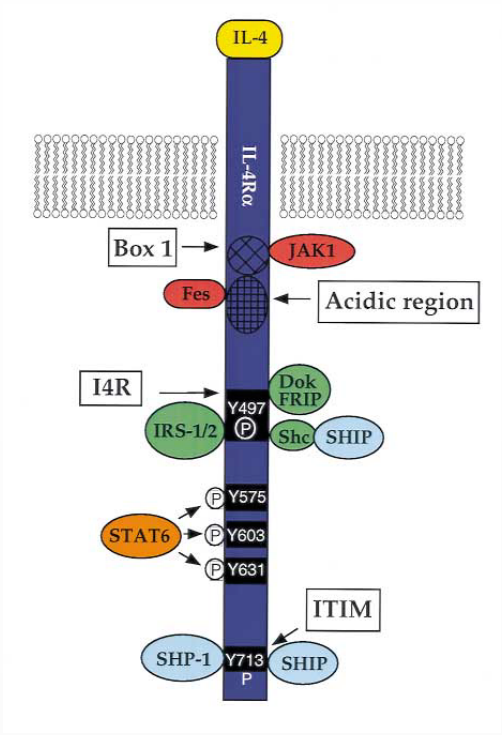
- Increased intestinal permeability
- Increased Goblet cell hyperplasia

SI antigen passage patterning is altered in *Il4ra*^{F709} mice ?

Il4ra^{F709}

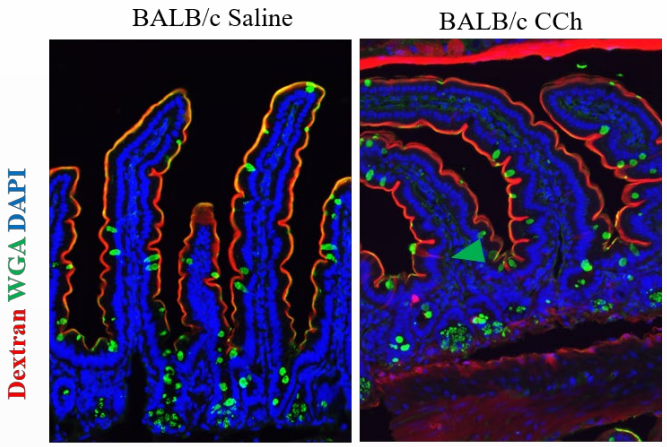
Do they have GAPs or SAPs ?

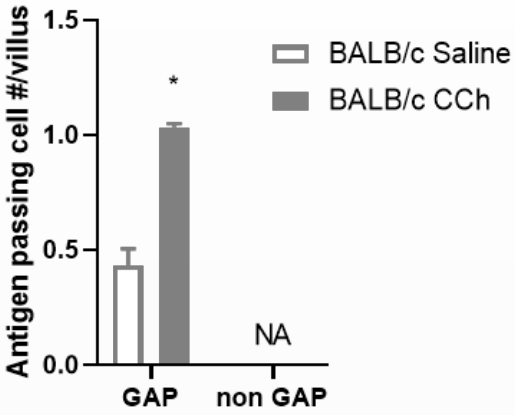
IL-4 and IL-13

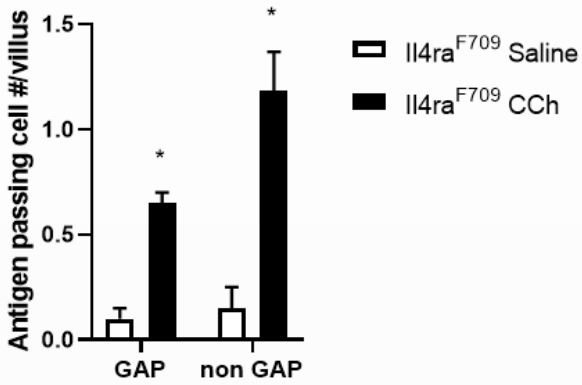


Noah et al., Front Immunol 2021 PMID: 33841417

SI antigen passage patterning is altered in *Il4ra*^{F709} mice



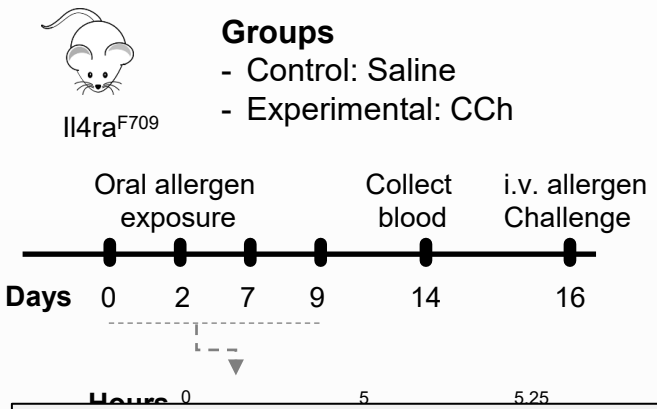


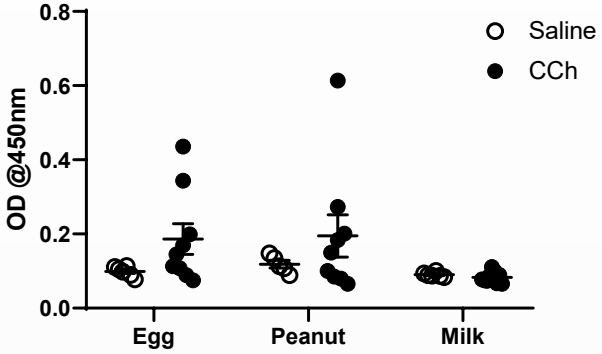


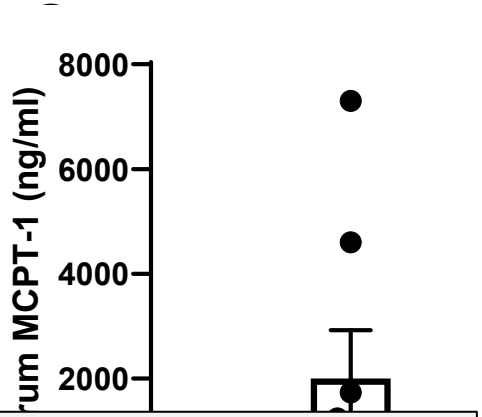
***SI Il4ra*^{F709} mice possesses SAPs at steady state**

Noah et al., Front Immunol 2021 PMID: 33841417

Steady State SI SAPs in *Il4ra*^{F709} mice







***SI Il4ra*^{F709} mice possesses SAPs at steady state**
Activation of SAPs in the presence of food allergens stimulates food sensitization

Steady State SI SAPs in *Il4ra*^{F709} mice promotes food sensitization and reactivity

Noah et al., Front Immunol 2021 PMID: 33841417

Human Tissue- SAP's

nature biomedical engineering

ARTICLES

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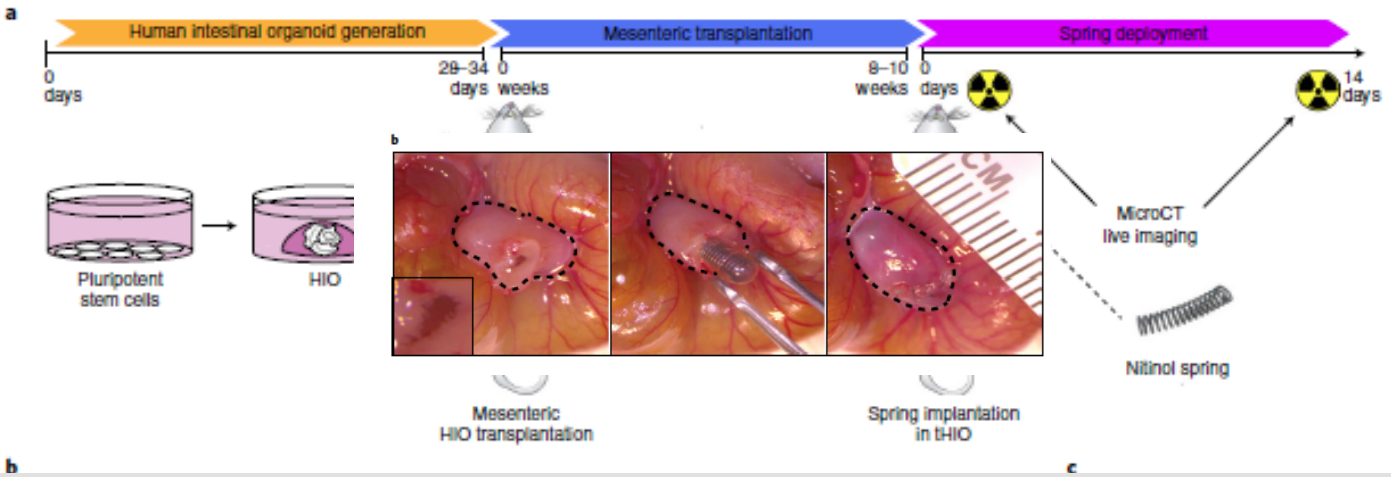
Mechanically induced development and maturation of human intestinal organoids in vivo

Holly M. Poling[1], David Wu[2], Nicole Brown[1], Michael Baker[3], Taylor A. Hausfeld[1], Nhan Huynh[4,5], Samuel Chaffron[6], James C. Y. Dunn[4,5,7,10], Simon P. Hogan[2], James M. Wells[8,9], Michael A. Helmrath[1] and Maxime M. Mahe[1,11]*

The natural ability of stem cells to self-organize into functional tissue has been harnessed for the production of functional human intestinal organoids. Although dynamic mechanical forces play a central role in intestinal development and morphogenesis, conventional methods for the generation of intestinal organoids have relied solely on biological factors. Here, we show that the incorporation of uniaxial strain, using compressed nitinol springs, in human intestinal organoids transplanted into the mesentery of mice induces growth and maturation of the organoids. Assessment of morphometric parameters, transcriptome profiling and functional assays of the strain-exposed tissue revealed higher similarities to native human intestine, with regard to tissue size and complexity, and muscle tone. Our findings suggest that the incorporation of physiologically relevant mechanical cues during the development of human intestinal tissue enhances its maturation and enterogenesis.



Poling et al., PMCID: PMC6108544

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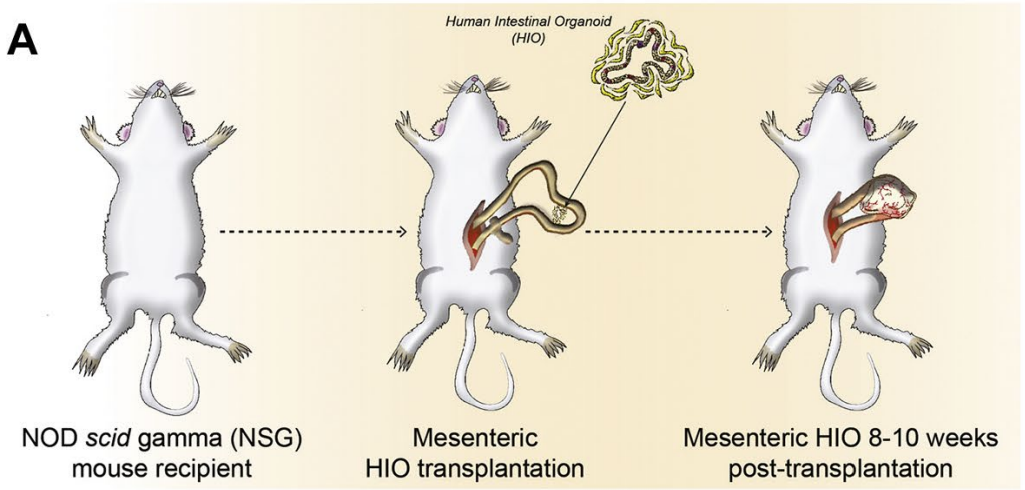

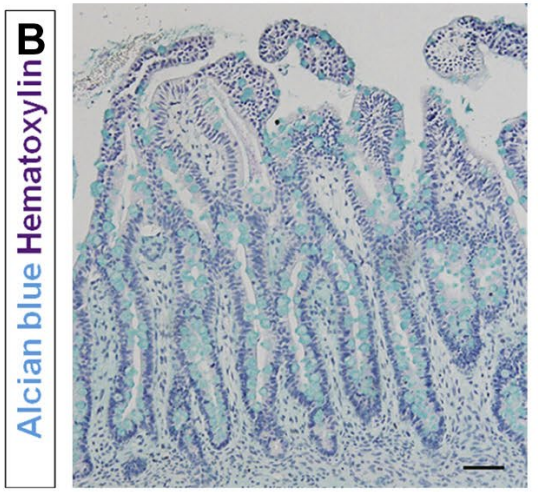

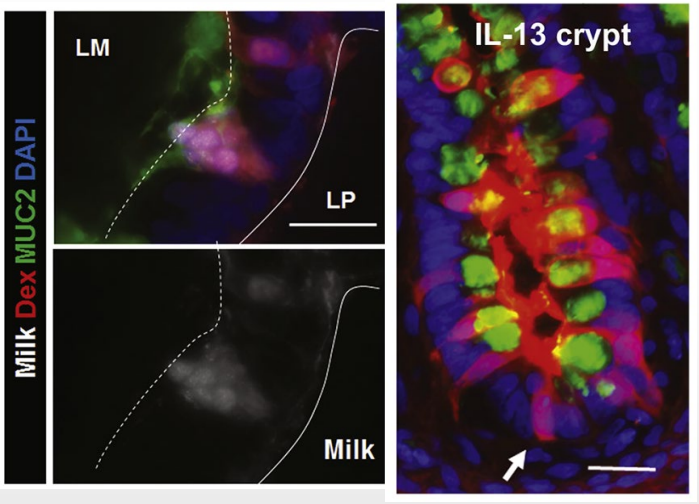

- **CCh-induced GAPs in tHIOs**
- **GAPs sample food allergens**
- **IL-13-induced SAPs in tHIOs**

Noah et al., JACI 2019 PMID: 31175877

Conclusion

- Food antigens and allergens cross the intestinal epithelium via multiple epithelial – immune processes.
- SI antigen passage patterning and landscape is altered under food allergic conditions.
- Food allergens cross the intestinal epithelium via SAPs and stimulate IgE-mast cell mediated reactions.
- SAPs are regulated by IL4Rα-dependent processes
- Blockade of SAPs inhibits food allergic reaction.
- SAPs sample food allergens in naïve state
- Altered antigen passage patterning may be the mechanisms by which the intestinal epithelium promotes oral food sensitization.
- Human intestinal tissue can generate GAPs and SAPs

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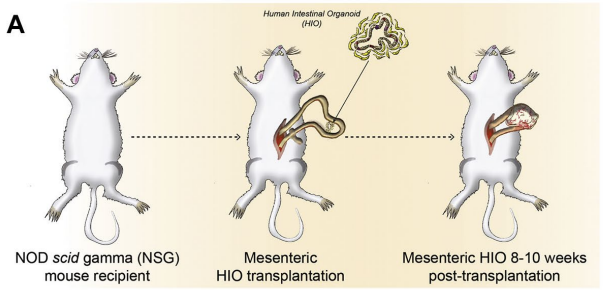

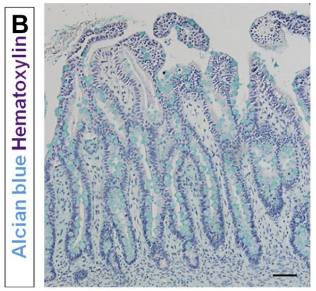

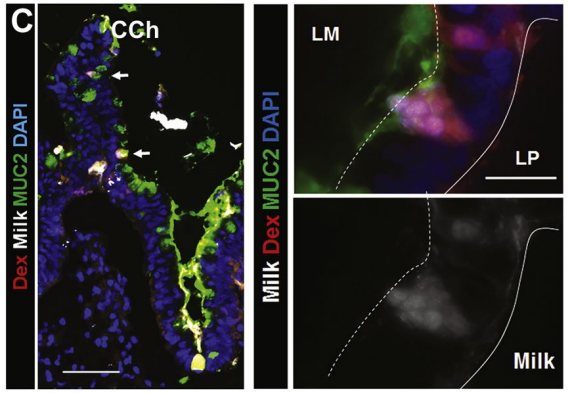

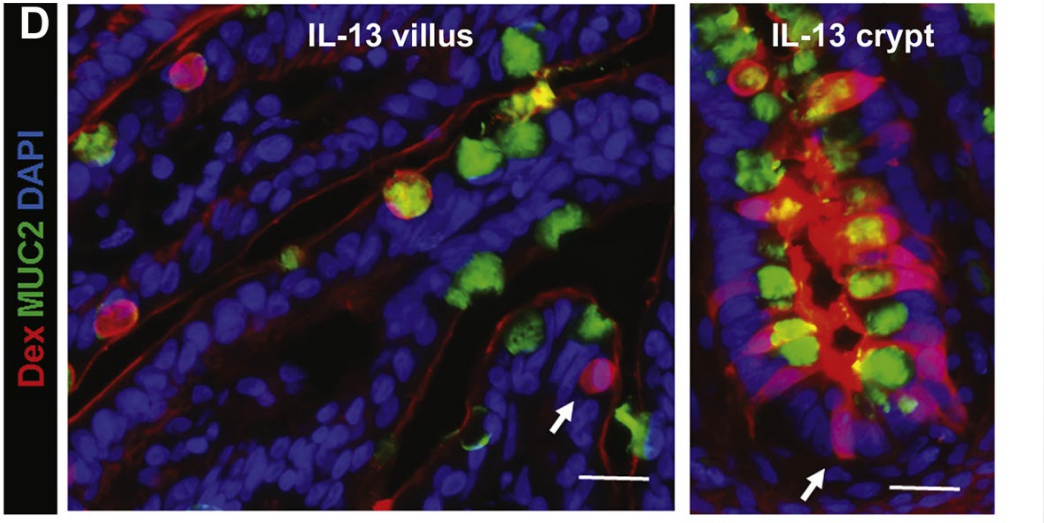

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