

# Links between allergy and gut function



Simon Murch

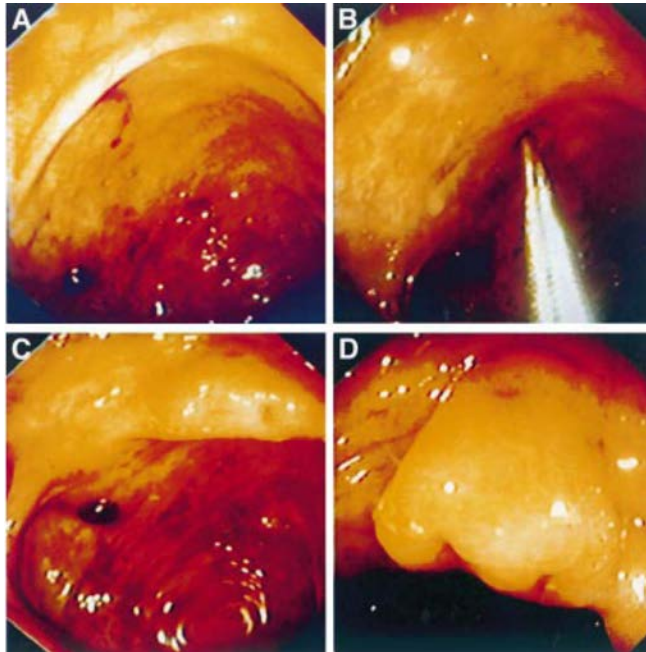
# GI food allergies

- May be of immediate, intermediate or delayed onset
  - IgE-mediated
  - Non-IgE-mediated – Th2 T cell, eosinophil
  - Mixed IgE and non-IgE-mediated
- May be part of systemic response or localised
  - Separate gut compartment
  - May disrupt motility, cause visceral hyperalgesia

# Colonoscopic allergen provocation (COLAP): a new diagnostic approach for gastrointestinal food allergy

*Gut* 1997; 40: 745–753

S C Bischoff, J Mayer, J Wedemeyer, P N Meier, G Zeck-Kapp, B Wedi, A Kapp, Y Cetin,  
M Gebel, M P Manns



**Responses dependent on patient history,  
not on SPT or specific IgE**

**+ve COLAP seen in some cases with  
negative DBPCFC**

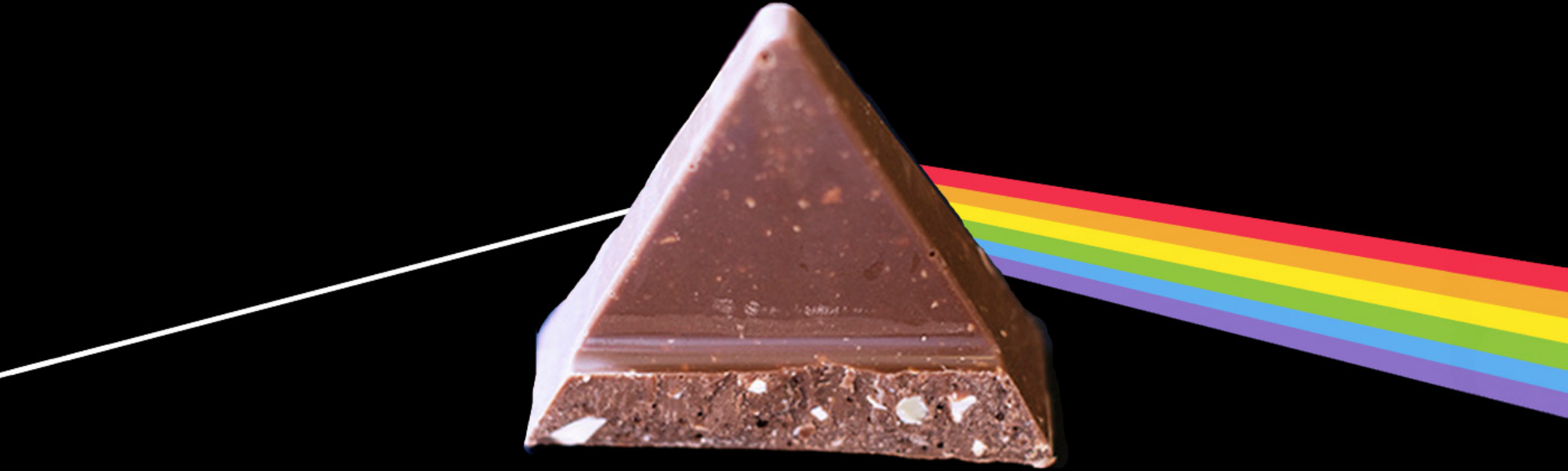


**Induced mast cell  
degranulation**

# Causes of food allergy

- Impaired oral tolerance mechanisms
  - Loss of previously acquired tolerance
    - Often pathogens break epithelial barrier
      - eg Cow's milk allergy after rotavirus
    - Secondary sensitisation to soya etc
  - Failure to establish oral tolerance initially
    - Immunological abnormalities
    - Inadequate innate immune exposures
      - eg breast-milk sensitisation, multiple food allergy

NON IGE  
MEDIATED  
THE  
DARK SIDE  
OF FOOD  
ALLERGY



# Non-IgE-mediated allergies

## ● Systemic manifestations

- Eczema, Asthma

## ● Localised manifestations

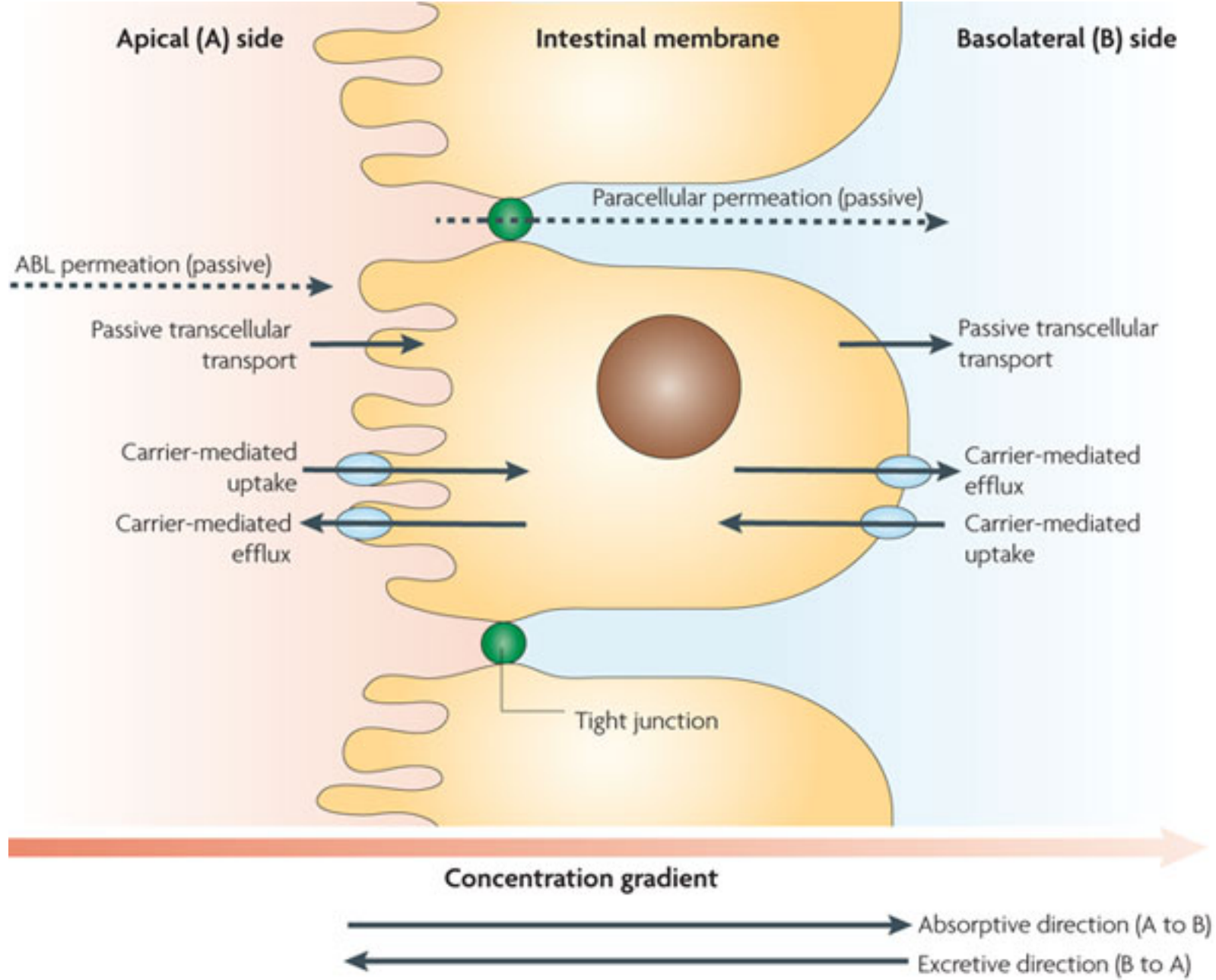
- Allergic/eosinophilic oesophagitis
- Small intestinal enteropathy: CMSE etc
- Colitis – Microscopic, milk-induced, FPIES
- Eosinophilic proctitis + constipation
- Dysmotility syndromes (Infant colic, IBS)

# Epithelial leakiness in allergic disease

- Appears to be consistent feature when tested
  - However tests have innate limitations
    - Lactulose-mannitol absorption most common
  - Bi-directional fluid flux
  - Albumin loss through reduced charged barrier
    - Faecal  $\alpha$ -1 antitrypsin most common marker
  - Increased antigen uptake through or round epithelial cells

# Indirect effects of carbohydrates on absorption and immune responses

- Glucose absorption via SGLT-1 increases paracellular permeability
  - Attisook and Madara 1991 – penetration of 11 aa oligopeptide during glucose absorption
- Unabsorbed sugars alter composition of gut flora
  - Important in enteropathy



**Identical intestinal permeability changes in children with different clinical manifestations of cow's milk allergy**

*J ALLERGY CLIN IMMUNOL 1991;88:737-42.*

Taina Jalonen, MD

**TABLE II.** Urinary recovery of test markers in patients with CMA before (day 0) and during milk challenge (day 3) and in control subjects

	Before cow's milk challenge (day 0)	During cow's milk challenge (day 3)	Paired Student's <i>t</i> test	
			<i>t</i>	<i>p</i>
Patients with skin symptoms (N = 24)				
Lactulose/mannitol ratio	0.02(0.01, 0.03)	0.06(0.03, 0.13)	2.76	0.01
Mannitol (%)	2.77(1.38, 5.56)	2.29(1.05, 5.01)	0.15	0.88
Lactulose (%)	0.05(0.02, 0.11)	0.14(0.06, 0.29)	1.69	0.11
Patients with GI symptoms (N = 27)				
Lactulose/mannitol ratio	0.02(0.01, 0.03)	0.08(0.04, 0.17)	2.88	0.01
Mannitol (%)	1.87(0.97, 3.62)	1.27(0.62, 2.60)	0.43	0.68
Lactulose (%)	0.03(0.01, 0.06)	0.09(0.03, 0.23)	2.92	0.01
Control subjects (N = 13)				
Lactulose/mannitol ratio		0.01(0.01, 0.02)		
Mannitol (%)		3.54(2.52, 4.96)		
Lactulose (%)		0.05(0.03, 0.08)		

Means (95% CI).

Weaning to Hypoallergenic Formula Improves Gut Barrier  
Function in Breast-Fed Infants With Atopic Eczema

*JPGN* 38: 92–96, 2004.

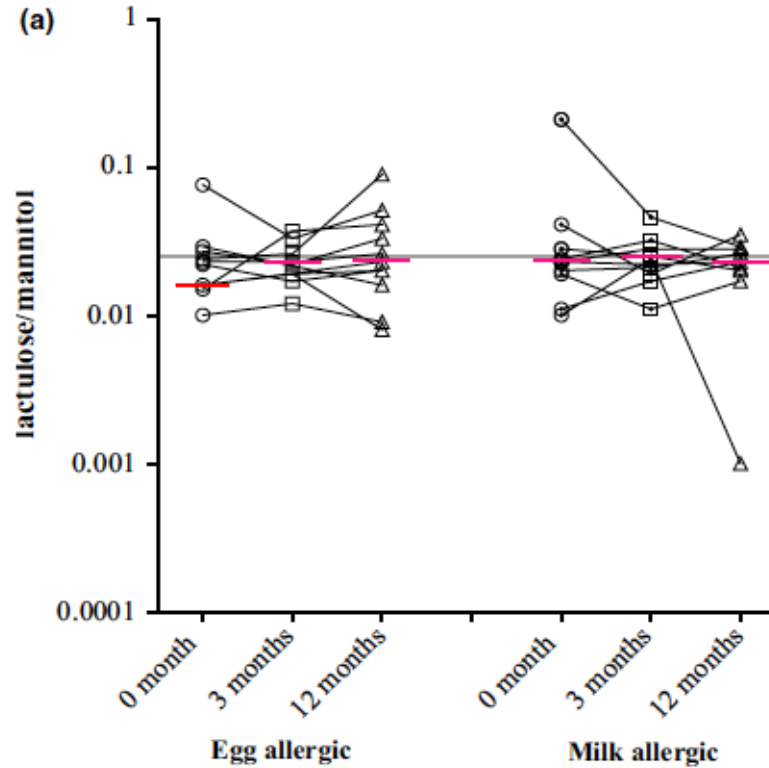
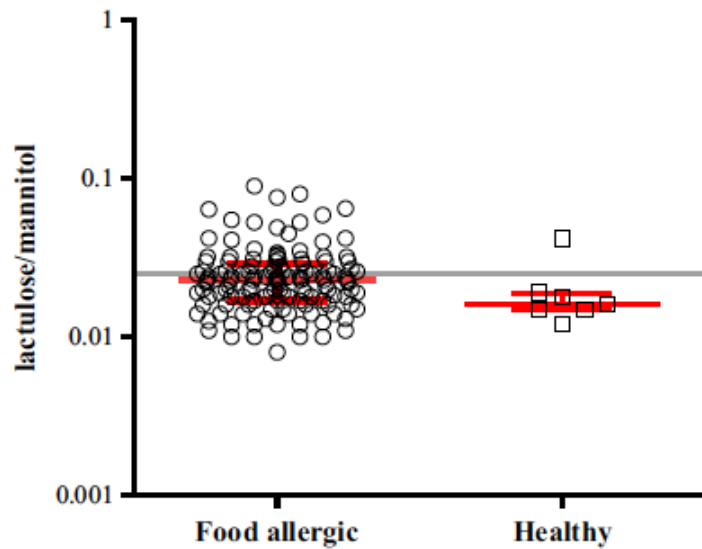
\*Taina Arvola, †Eeva Moilanen, ‡Risto Vuento, and §Erika Isolauri

**TABLE 1.** Study parameters of infants with atopic eczema ( $n = 56$ ) during breast-feeding and 2 and 7 months after weaning

	During breastfeeding	2 Months after weaning	7 Months after weaning	<i>P</i> value
SCORAD*	22 (18–27)	10 (6–13)	5 (3–7)	<0.00001‡
Urinary eosinophil protein X concentration ( $\mu\text{g}/\text{mmol}$ creatinine)†	101.6 (67.1–171.7)	58.8 (33.6–114.0)		0.003
Urinary methylhistamine concentration ( $\mu\text{g}/\text{mmol}$ creatinine)†	52.0 (41.2–67.9)	53.0 (45.4–66.6)		0.38
Urinary recovery ratio of lactulose and mannitol†	0.029 (0.021–0.042)	0.023 (0.016–0.031)		0.0006
Fecal $\alpha$ -1 antitrypsin concentration (mg/g)†	2.3 (1.2–3.3)	0 (0.0–1.9)	0.8 (0.0–1.2)	<0.0001(3)

# Intestinal permeability in children with food allergy on specific elimination diets

Kirsi M. Järvinen<sup>1,2</sup>, George N. Konstantinou<sup>2,3</sup>, Mariecel Pilapil<sup>2</sup>, Marie-Claire Arrieta<sup>4</sup>, Sally Noone<sup>2</sup>, Hugh A. Sampson<sup>2</sup>, Jon Meddings<sup>5</sup> & Anna Nowak-Węgrzyn<sup>2</sup>



**1/3 showed persistent increased permeability on exclusion diet – associated with shorter stature**

**Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. Kalach N et al, Clin Chem Lab Med 2012.**

● 25 children with ? CMA mean age 6.3 months

■ Specific IgE/ IgG cow milk protein, SPT, atopy patch test and oral open cow's milk challenge.

■ Fecal microbiota, intestinal permeability (urinary L/M ratio), fecal  $\alpha$ 1-AT, TNF- $\alpha$ , calprotectin,  $\beta$ -defensin2, secretory IgA and EDN.

● Challenge + 11/25, - 14/25

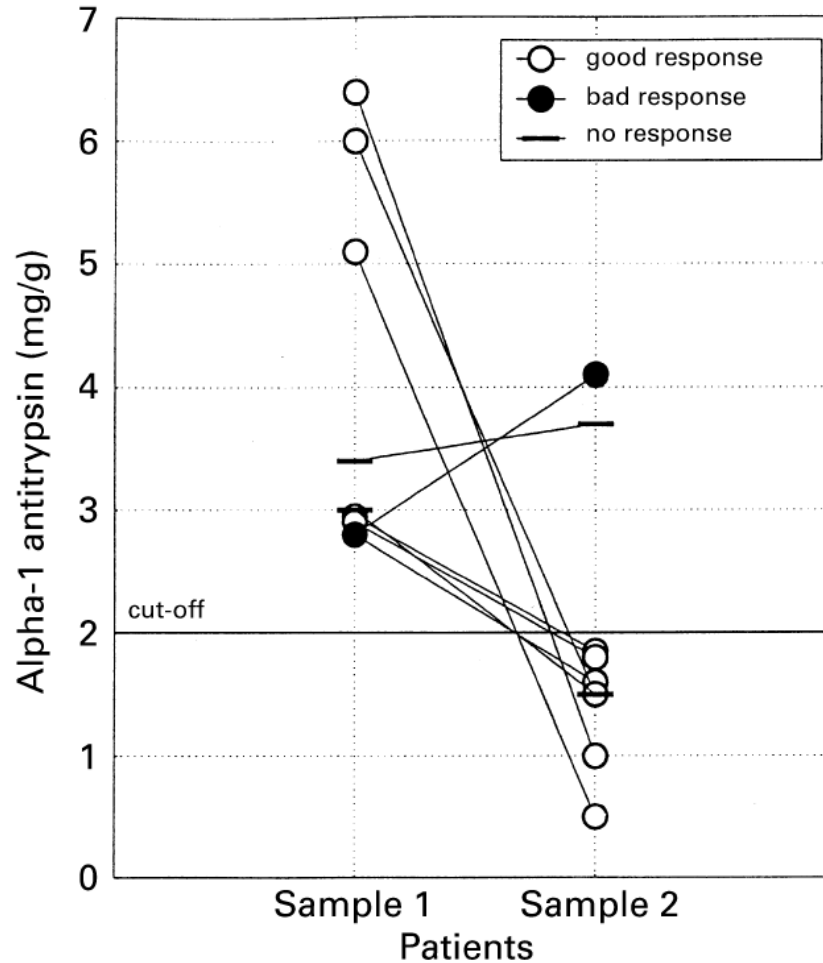
■ True positive + negative cases/total no

■ 76% for intestinal permeability; 72% for fecal EDN; contrasting with atopy patch test, 68%; IgE, 60%; skin prick test, 55% and IgG, 52%.

# Increased concentration of fecal $\alpha_1$ -antitrypsin is associated with cow's milk allergy in infants with atopic eczema

*Clinical and Experimental Allergy*, 2001, Volume 31, pages 590–592

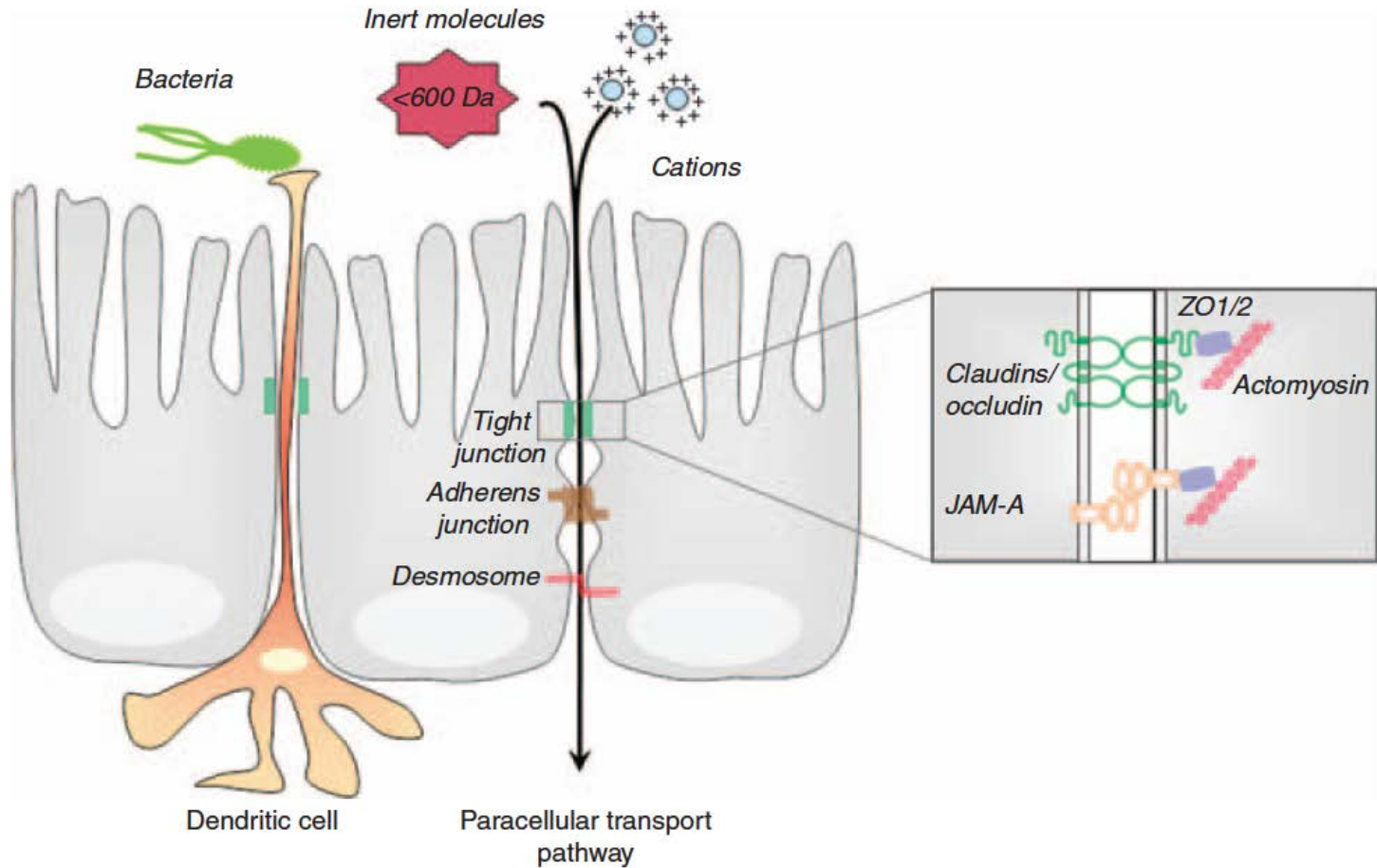
H. MAJAMAA\*†, J. AITTONIEMI‡ and A. MIETTINEN‡



# Intestinal permeability

- Largely determined by paracellular leak
  - Tight junction integrity
  - Charge based selectivity (Claudins, heparan sulphate proteoglycans)
  - Enterocyte adhesion to basement membrane
- Transcellular permeability
  - Diffusion – small molecules
  - Active carrier mediated transport
  - Transcytosis

# Paracellular transport pathways



Multiple facets of intestinal permeability and epithelial handling of dietary antigens

S Ménard<sup>1</sup>, N Cerf-Bensussan<sup>1</sup> and M Heyman<sup>1</sup>

Mucosal Immunology | VOLUME 3 NUMBER 3 | MAY 2010

# Regulators of tight junctions

- Claudin family – knockout mice have leaky epithelia
- Junctional adhesion molecule A (JAM-A)
  - Decreased JAM-A reported in IBD
- Occludin
- Tricellulin
- Zonulin

# Constitutive GI permeability increase?

- No clear evidence
  - Milk allergy biopsies show ↑ HRP transport
  - However normalise on exclusion diet
- Environmental factors ↑ susceptibility
  - eg infection, stress
- IBD ↑ GI permeability in 1<sup>st</sup> degree relatives
  - Genetic studies – ? Regulators of barrier function
    - IBD5, DLG5, PGTER4, ITLN1, DMBT1, XBP1

# Induction of enhanced paracellular permeability

- Pathogens
  - Direct cytotoxic effects
  - Toxins
- Cytokines
  - IFN- $\gamma$ , TNF- $\alpha$   $\uparrow$
  - TGF- $\beta$ , IL-10  $\downarrow$
- Endogenous mediators
  - ZO-1

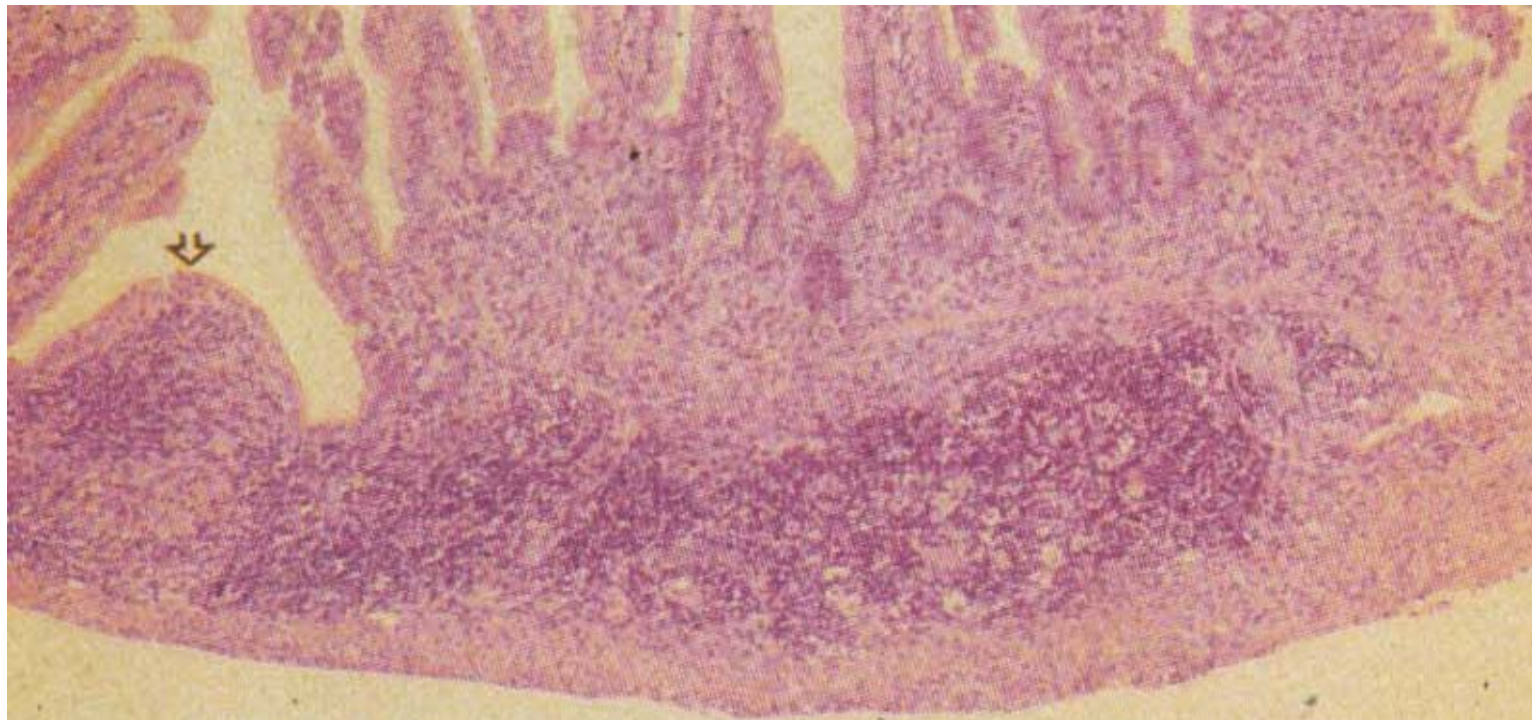
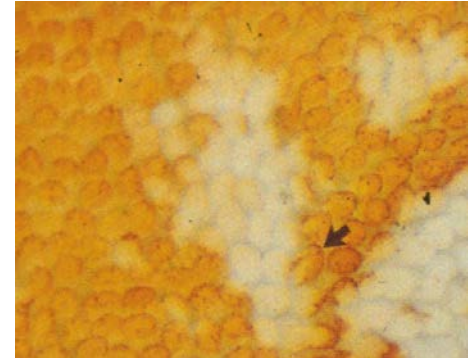
# Effect of proinflammatory cytokines

- ↑ both transcellular and paracellular transport
  - May also induce ulceration or apoptosis
    - IL-13 promotes enterocyte apoptosis
  - Effect myosin light chain kinase – open TJ's
  - May redistribute TJ molecules (IFN- $\gamma$ , TNF- $\alpha$ )
    - Leads to increased transcytosis of macromolecules

# Increased permeability causes inflammation

**Focal mutation of epithelial cadherins causes transmural inflammation.**

**Hermiston and Gordon , *Science* 1995**



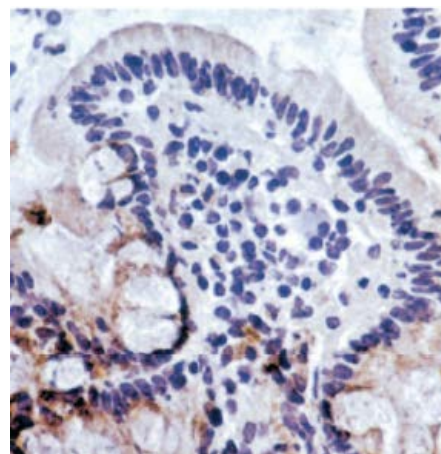
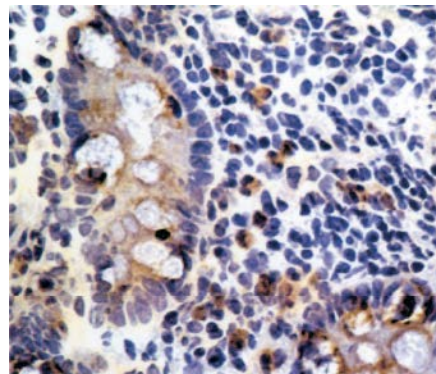
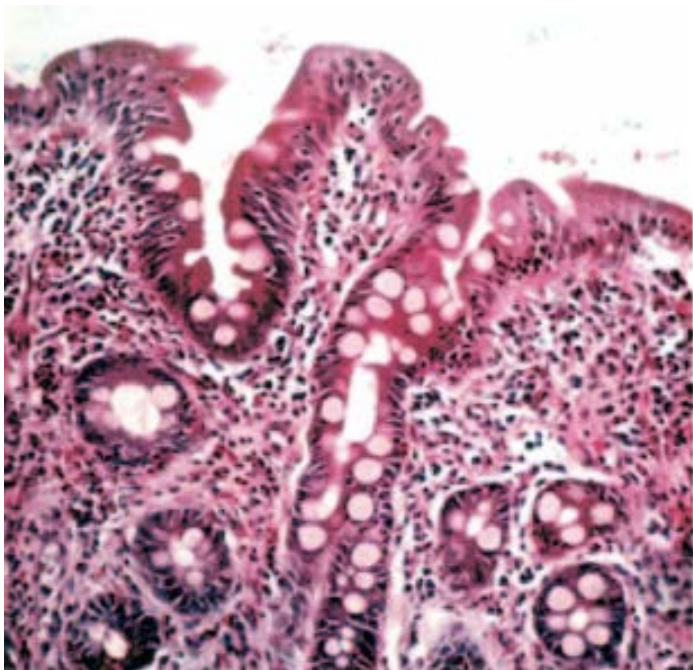
# A global perspective

- Increased LM ratio major predictor of mortality in Gambian infants (Lunn 1991).
- LM ratio (mean 0.38) worsened by nutritional rehabilitation (Sullivan 1992)
- Severity of marasmus associated with failed mucosal TGF- $\beta$  response (Campbell 2003)
- Amino Acid formula feeding allowed significantly greater weight gain than isocaloric lactose-free cow milk in malnourished Zambian infants (Amadi 2005)

# Chronic T Cell–Mediated Enteropathy in Rural West African Children: Relationship with Nutritional Status and Small Bowel Function

DAVID I. CAMPBELL, SIMON H. MURCH, MARINOS ELIA, PETER B. SULLIVAN, MUSTAPHA S. SANYANG, BABA JOBARTEH, AND PETER G. LUNN

*Pediatr Res* 54: 306–311, 2003



TGF- $\beta$  cells

**Worsening nutritional status associated with failure of regulatory immune response and dominance of pro-inflammatory mechanisms**

# Improved Nutritional Recovery on an Elemental Diet in Zambian Children with Persistent Diarrhoea and Malnutrition

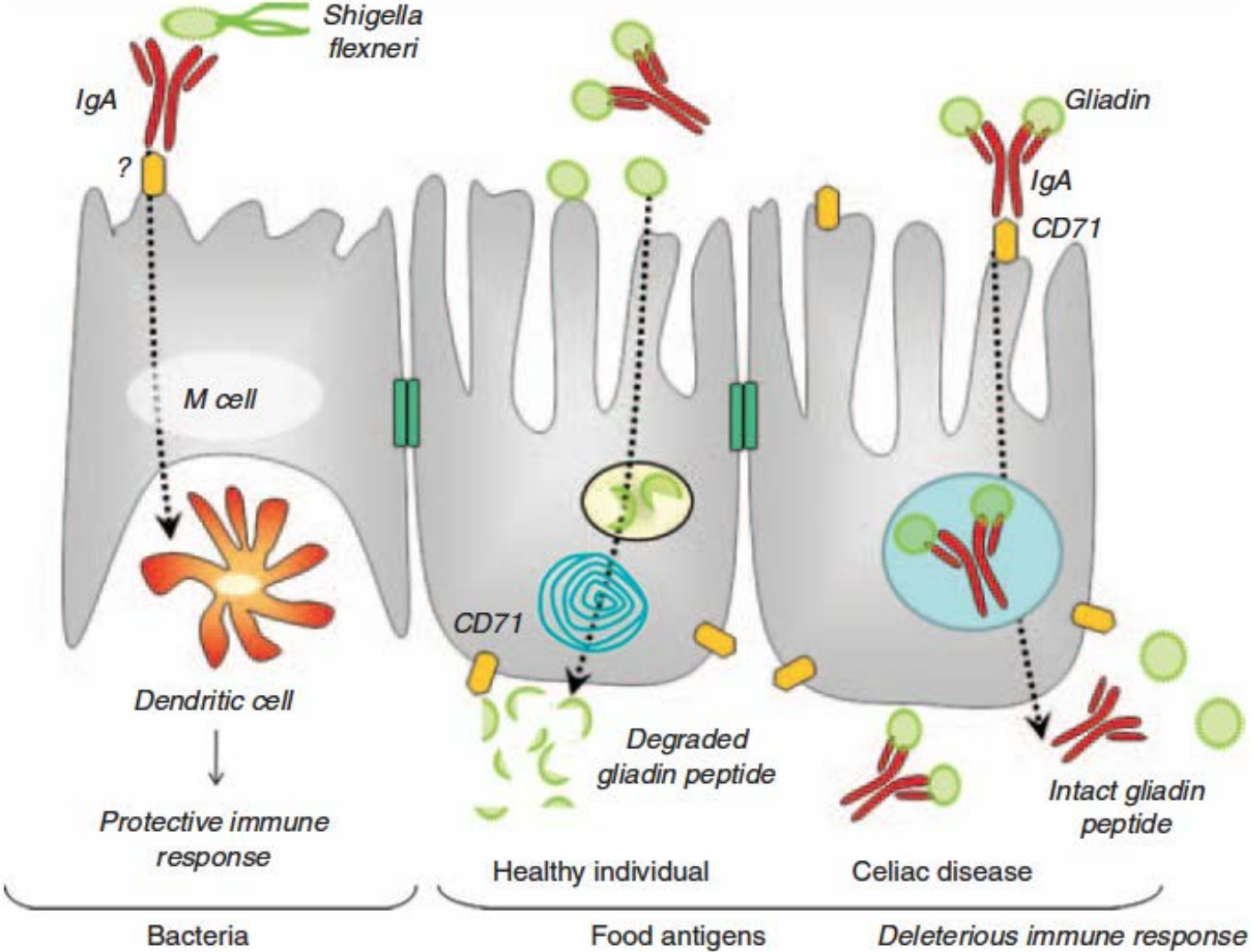
Journal of Tropical Pediatrics 2005;

Beatrice Amadi,<sup>a,b</sup> Mwiya Mwiya,<sup>a</sup> Elwyn Chomba,<sup>a</sup> Mike Thomson,<sup>c</sup> Chifumbe Chintu,<sup>a</sup> Paul Kelly,<sup>b,d</sup> and John Walker-Smith<sup>c</sup>

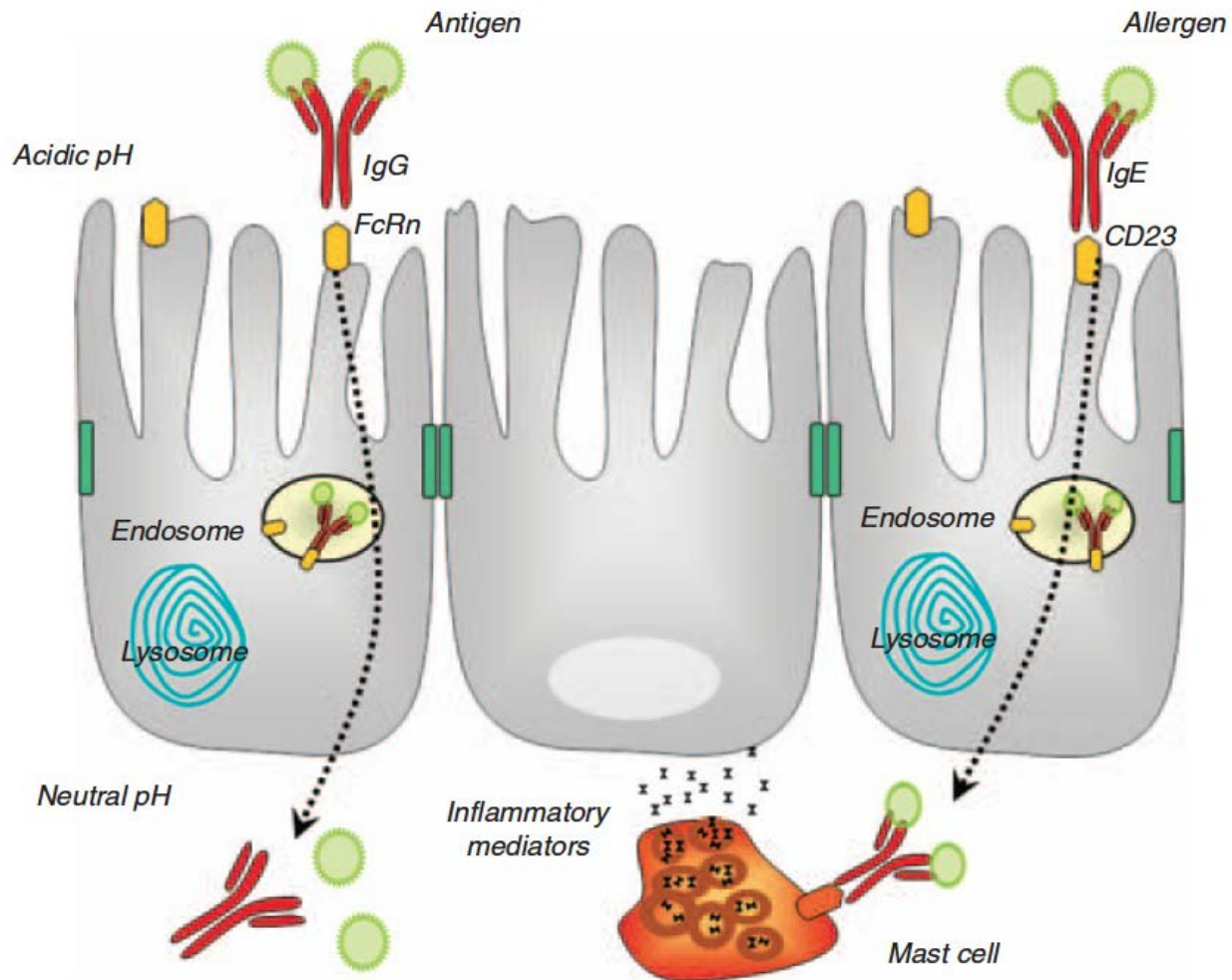
## *Nutritional recovery and treatment allocation group*

	Neocate	Control	<i>p</i>
<i>Weight gain (kg)</i>			
<i>n</i>	79	78	
From admission	1.10 (0.55–1.55)	0.75 (0.2–1.3)	0.006
From nadir	1.7 (1.2–2.0)	1.2 (0.6–1.7)	0.002
<i>Increase in weight-for-age z score</i>			
<i>n</i>	79	78	
From admission	0.83 (0.35–1.22)	0.43 (0–0.9)	0.018
From nadir	1.23 (0.89–1.57)	0.87 (0.47–1.25)	0.002
<i>Increase in weight-for-height z score</i>			
<i>n</i>	79	78	
From admission	1.28 (0.52–1.88)	0.56 (0–1.15)	<0.001
From nadir	1.77 (1.30–2.26)	1.23 (0.59–1.70)	<0.001

# Apical-basal transport – IgA mediated



# Apical-basal transport – IgG or IgE mediated



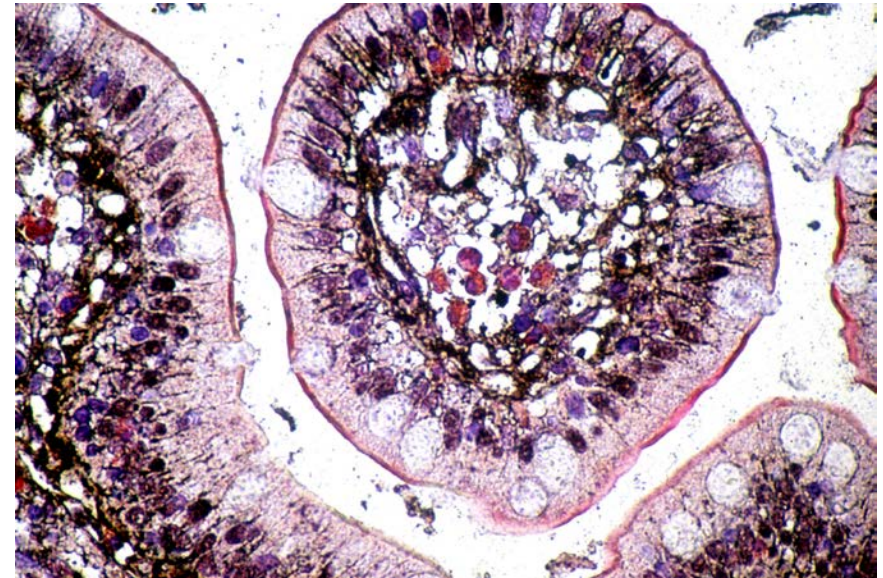
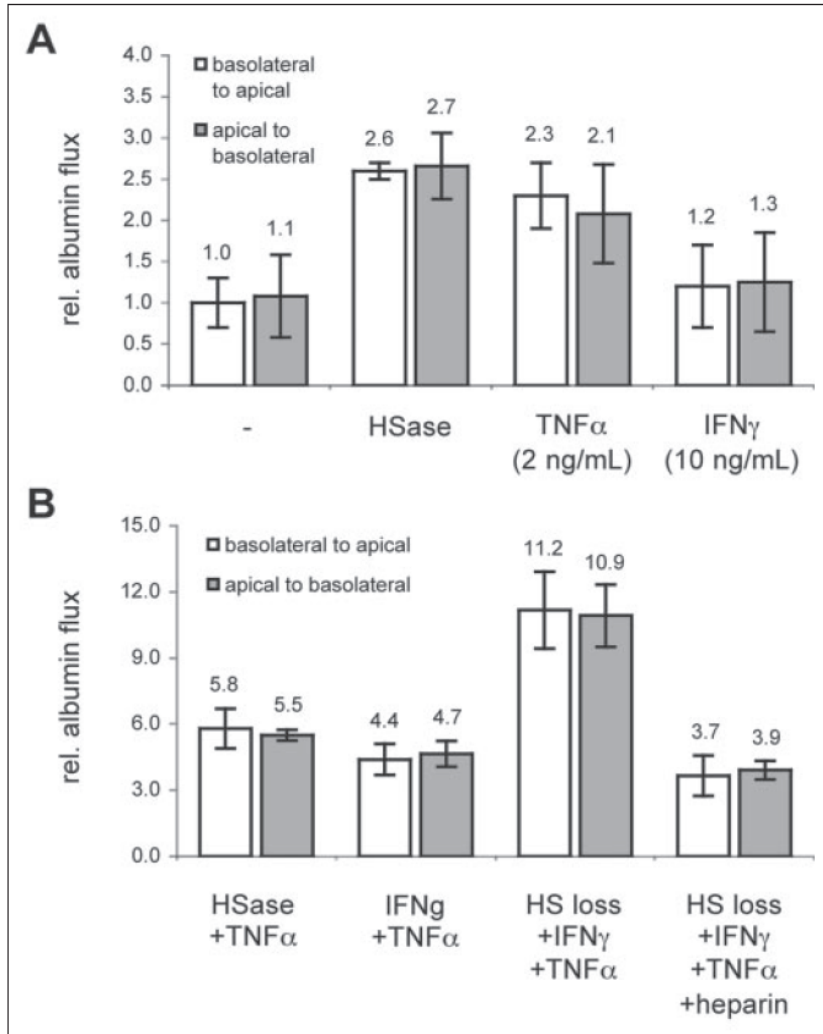
# Mechanism of albumin leak

- Acquired loss of –ve sulphated GAG barrier
  - Heparan sulphate proteoglycans
- Proinflammatory cytokines
- TNF- $\alpha$ , IFN- $\gamma$
- Hydrostatic pressure
- ? Genetic polymorphisms in GAG synthesis

# Heparan Sulfate Plays a Central Role in a Dynamic *in Vitro* Model of Protein-losing Enteropathy\*

Lars Bode<sup>‡</sup>, Simon Murch<sup>§</sup>, and Hudson H. Freeze<sup>‡1</sup>

THE JOURNAL OF BIOLOGICAL CHEMISTRY  
VOL. 281, NO. 12, pp. 7809–7815, March 24, 2006

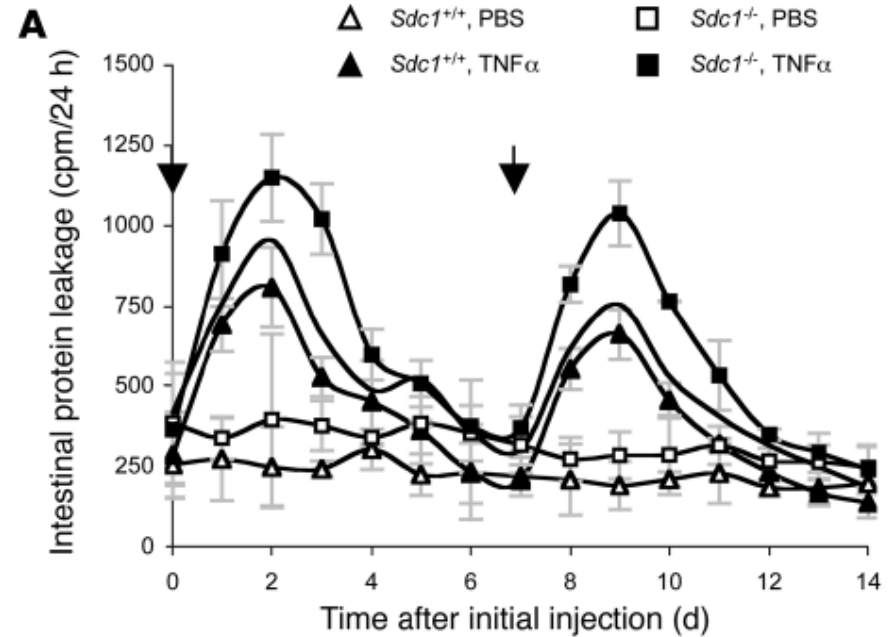
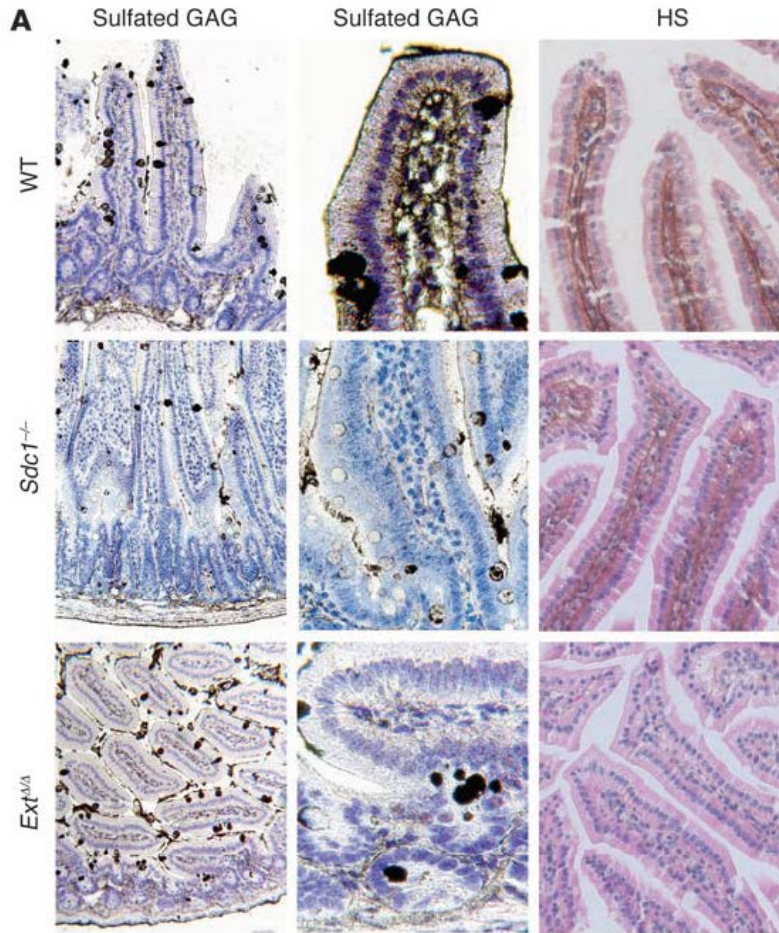


**The charged barrier within the lateral intercellular spaces may modulate antigen penetration**

# Heparan sulfate and syndecan-1 are essential in maintaining murine and human intestinal epithelial barrier function

Lars Bode,<sup>1</sup> Camilla Salvestrini,<sup>2</sup> Pyong Woo Park,<sup>3</sup> Jin-Ping Li,<sup>4</sup> Jeffrey D. Esko,<sup>5</sup> Yu Yamaguchi,<sup>1</sup> Simon Murch,<sup>6</sup> and Hudson H. Freeze<sup>1</sup>

*J. Clin. Invest.* **118**:229–238 (2008).



# Reduced production of sulfated glycosaminoglycans occurs in Zambian children with kwashiorkor but not marasmus

Beatrice Amadi, Andrew O Fagbemi, Paul Kelly, Mwiya Mwiya, Franco Torrente, Camilla Salvestrini, Richard Day,  
Michael H Golden, Erik A Eklund, Hudson H Freeze, and Simon H Murch

*Am J Clin Nutr* 2009;89:592–600.

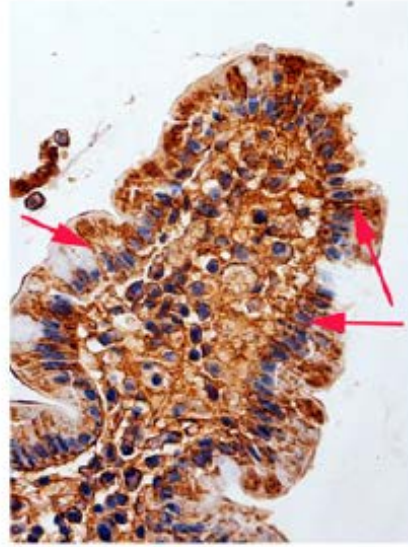
**A**

**GAGs**

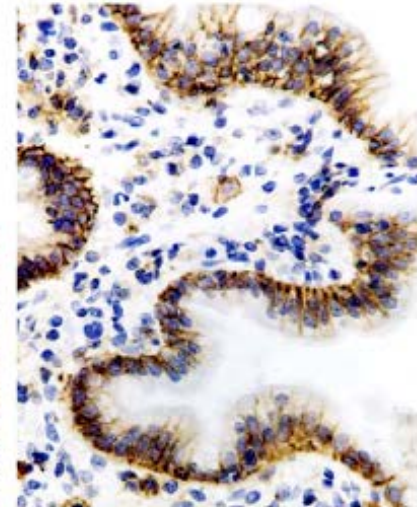
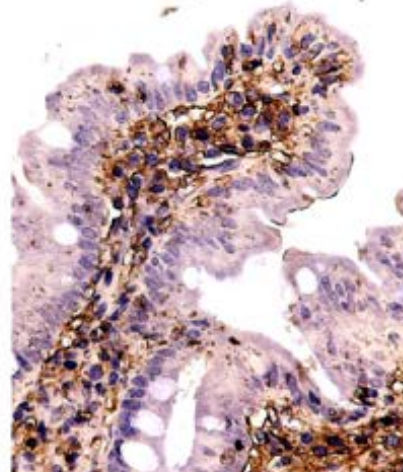
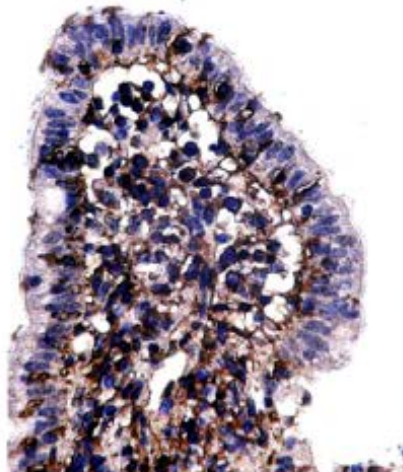
**HSPG**

**Syndecan-1**

**Maras**



**Kwash**



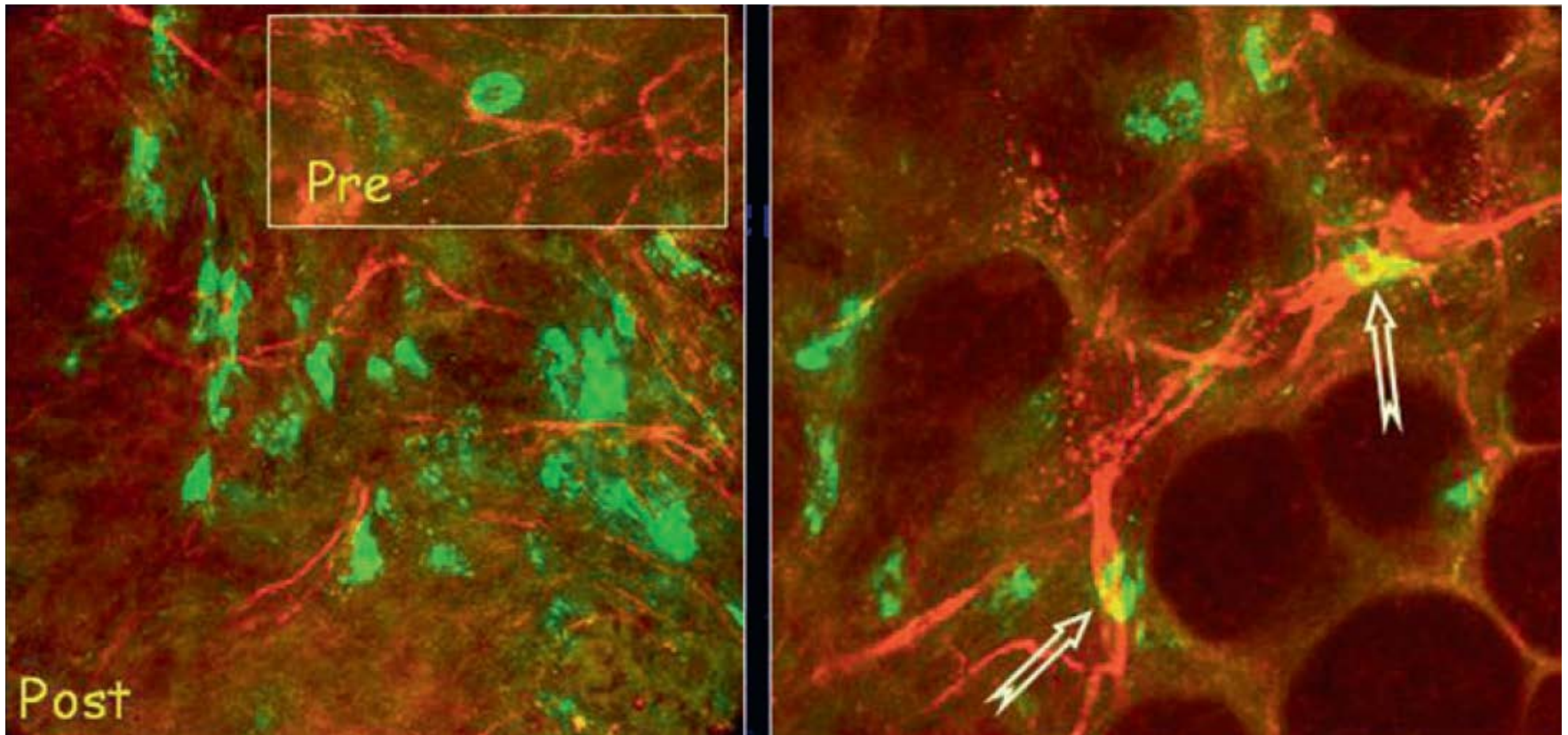
# Neurally-mediated hyperpermeability

- Dense innervation of mucosa
- Paracellular permeability regulated by neural activation
  - Marked induction of epithelial leakiness by psychological stress
  - Neural activation by mucosal immune responses

# Mast Cell–Nerve Interactions in Children With Functional Dyspepsia

*JPGN* 47:472–480, 2008.

\*Michela G. Schäppi, †Osvaldo Borrelli, ‡Daniela Knafelz, §Sue Williams,  
§Virpi V. Smith, ¶Peter J. Milla, and ¶Keith J. Lindley

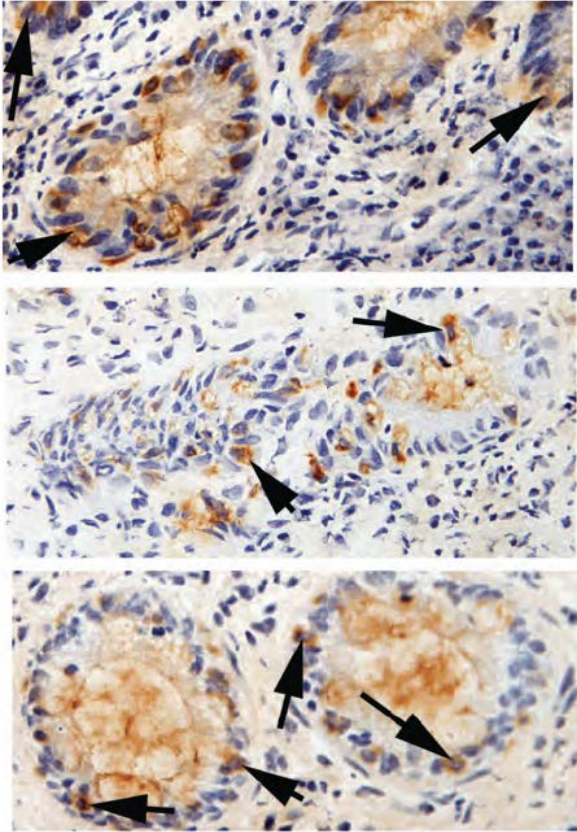


**Mast cell tryptase localises to nerve fibres after milk challenge  
performed during endoscopy**

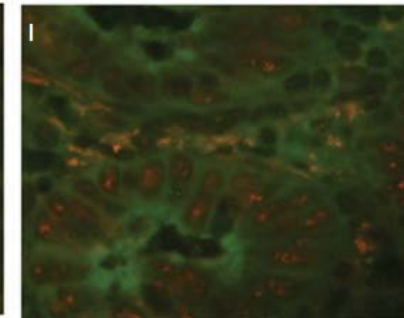
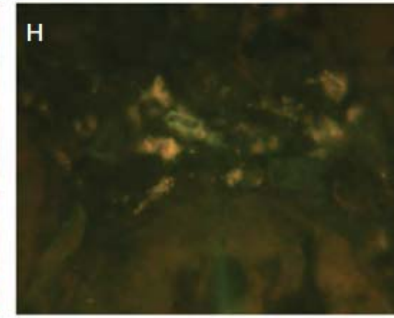
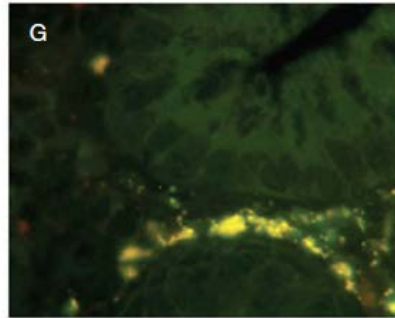
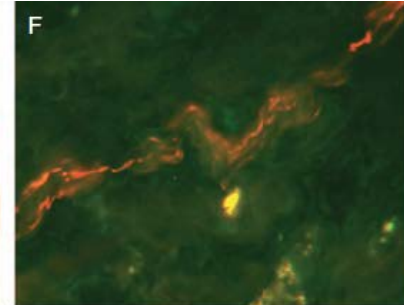
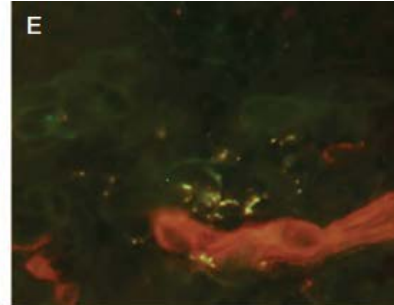
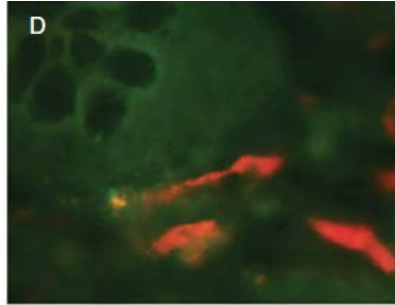
# Intraepithelial Lymphocyte Eotaxin-2 Expression and Perineural Mast Cell Degranulation Differentiate Allergic/Eosinophilic Colitis From Classic IBD

*JPGN* 2014;59: 300–307

\*Franco Torrente, †Arrigo Barabino, †Tommaso Bellini, and ‡Simon H. Murch



**Eotaxin-2 + IEL's**



**IgE and mast cell tryptase localise to enteric nerves**

# Summary

- Enhanced paracellular permeability common in intestinal food allergy
- No evidence yet for constitutive leaky gut
  - Possible genetic basis for acquired leak
- Sensitisation affects antigen ingress
  - Transcellular
  - Paracellular
- Subepithelial mast cell response important
  - Mixed Non-IgE mediated and localised IgE response may be common