Nutritional factors controlling intestinal immunity a pathologists view

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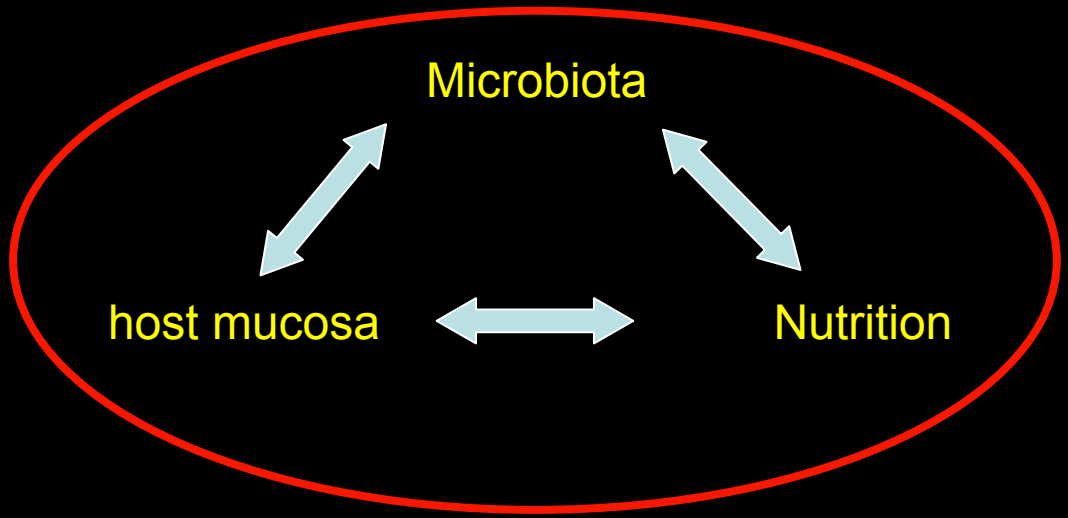
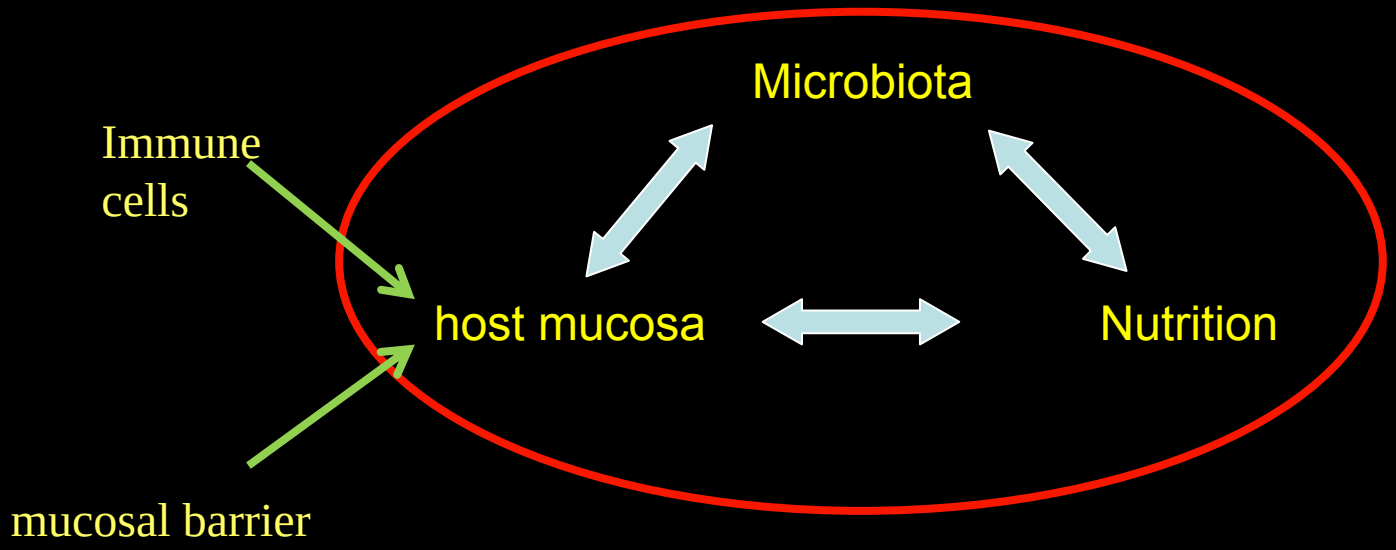
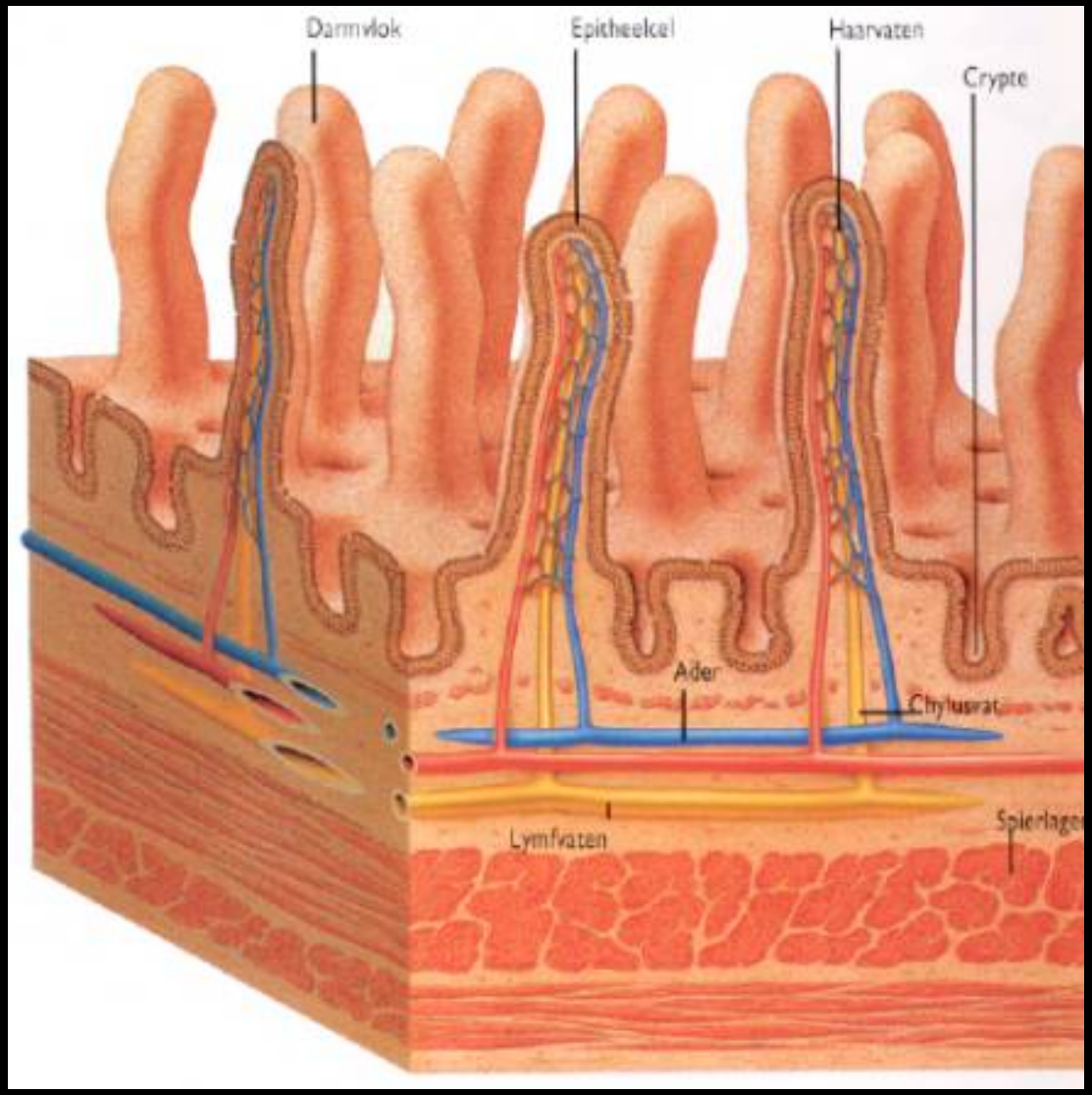
Faculty of Veterinary Medicine

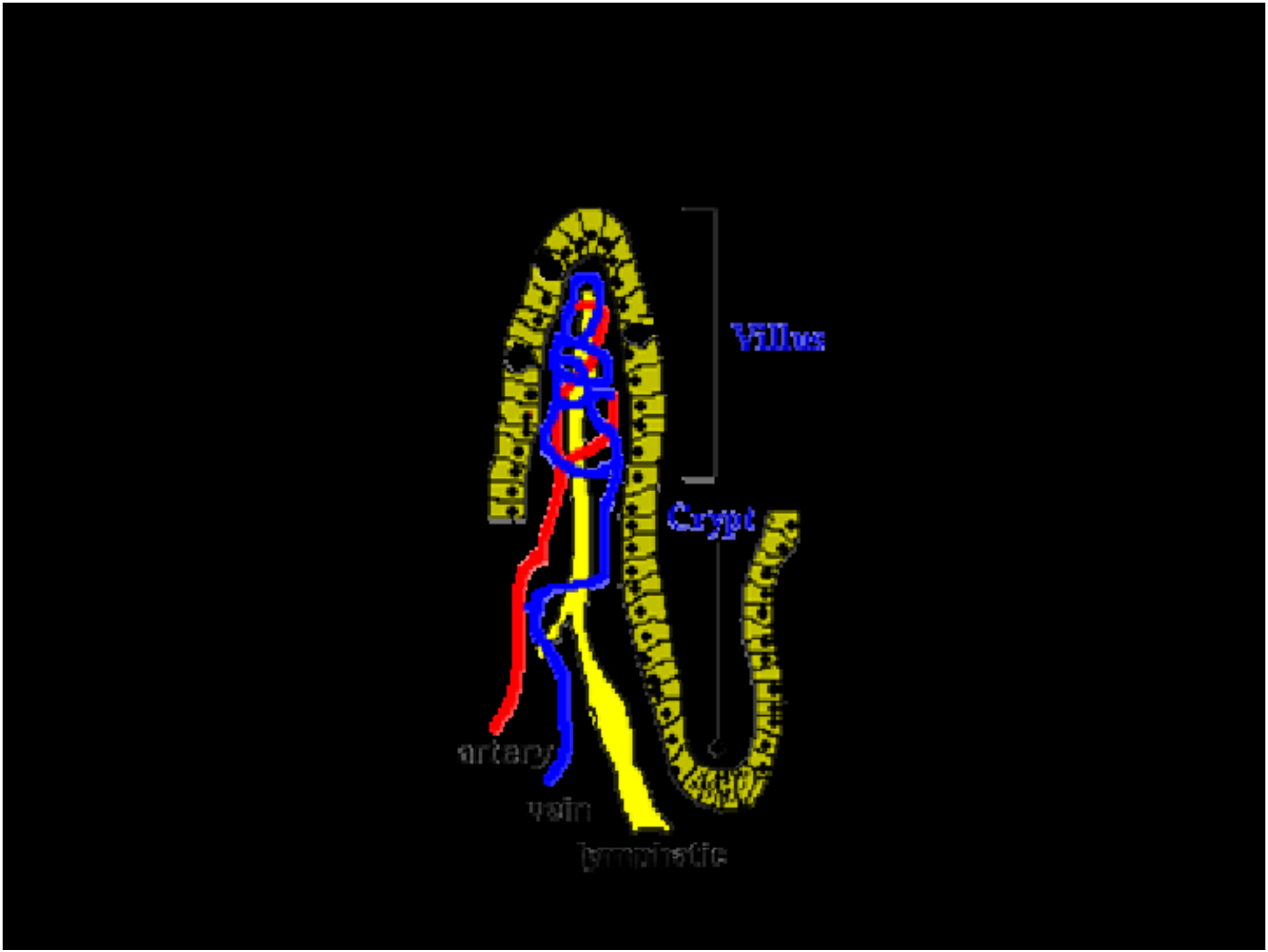
The intestinal ecosystem

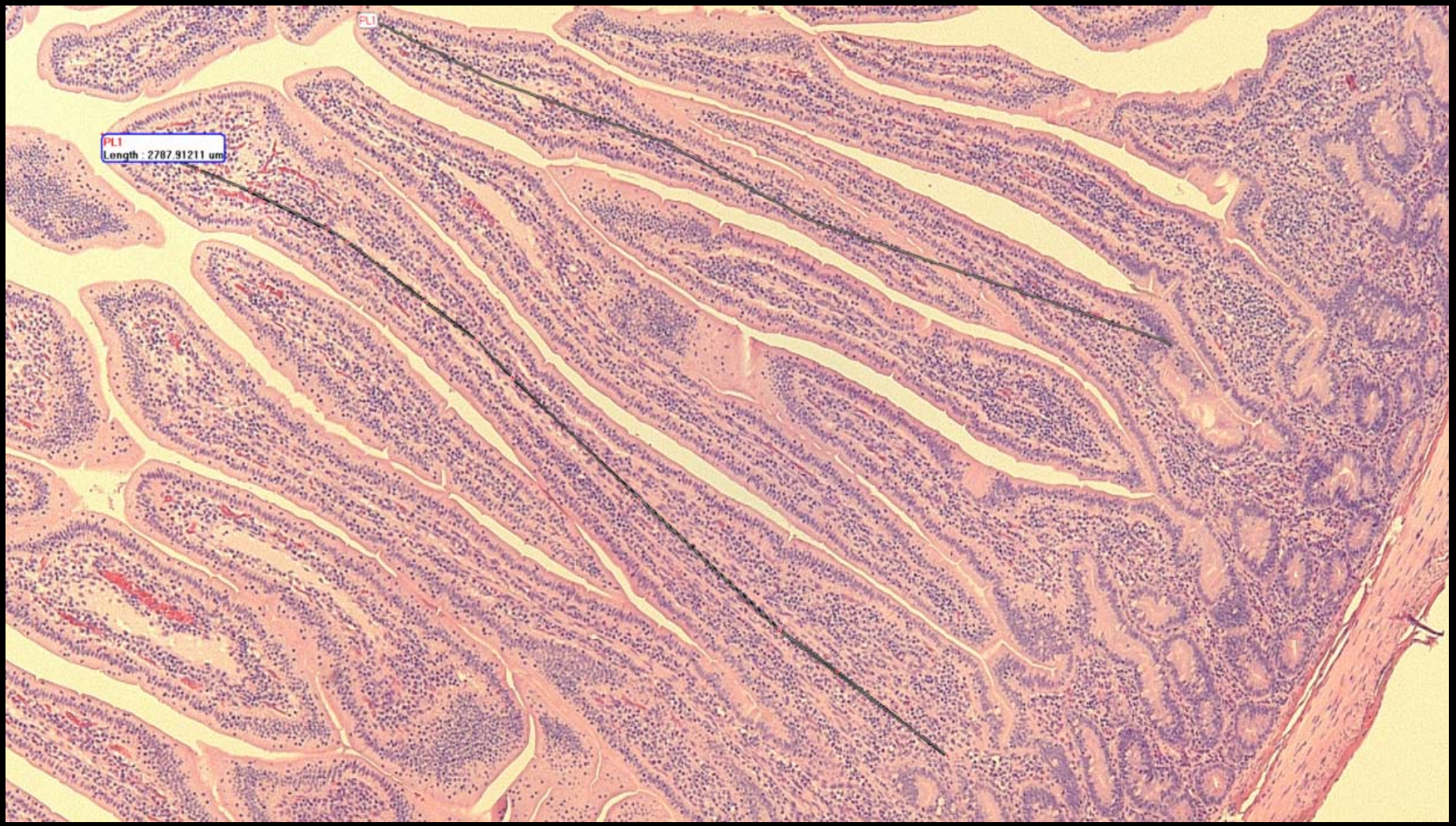


The intestinal ecosystem




Darmvlok
Epitheelcel
Haarvaten
Crypte
Ader
Chylusvat
Lymfvaten
Spierlaag

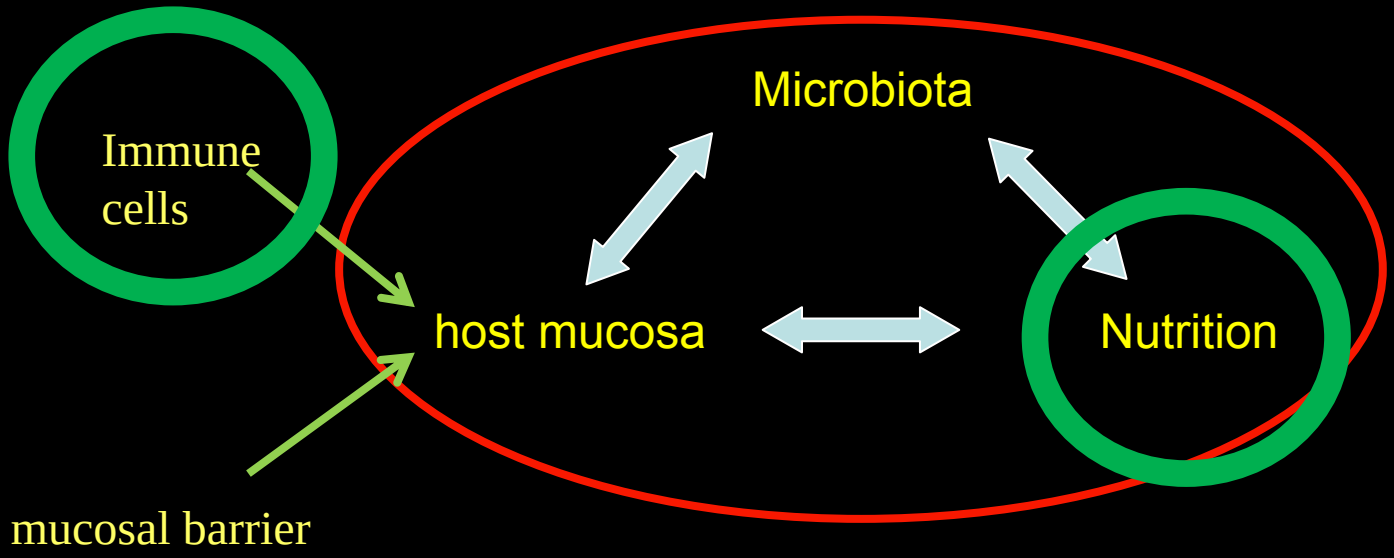

Villus
Crypt
artery
vein
lymphatic

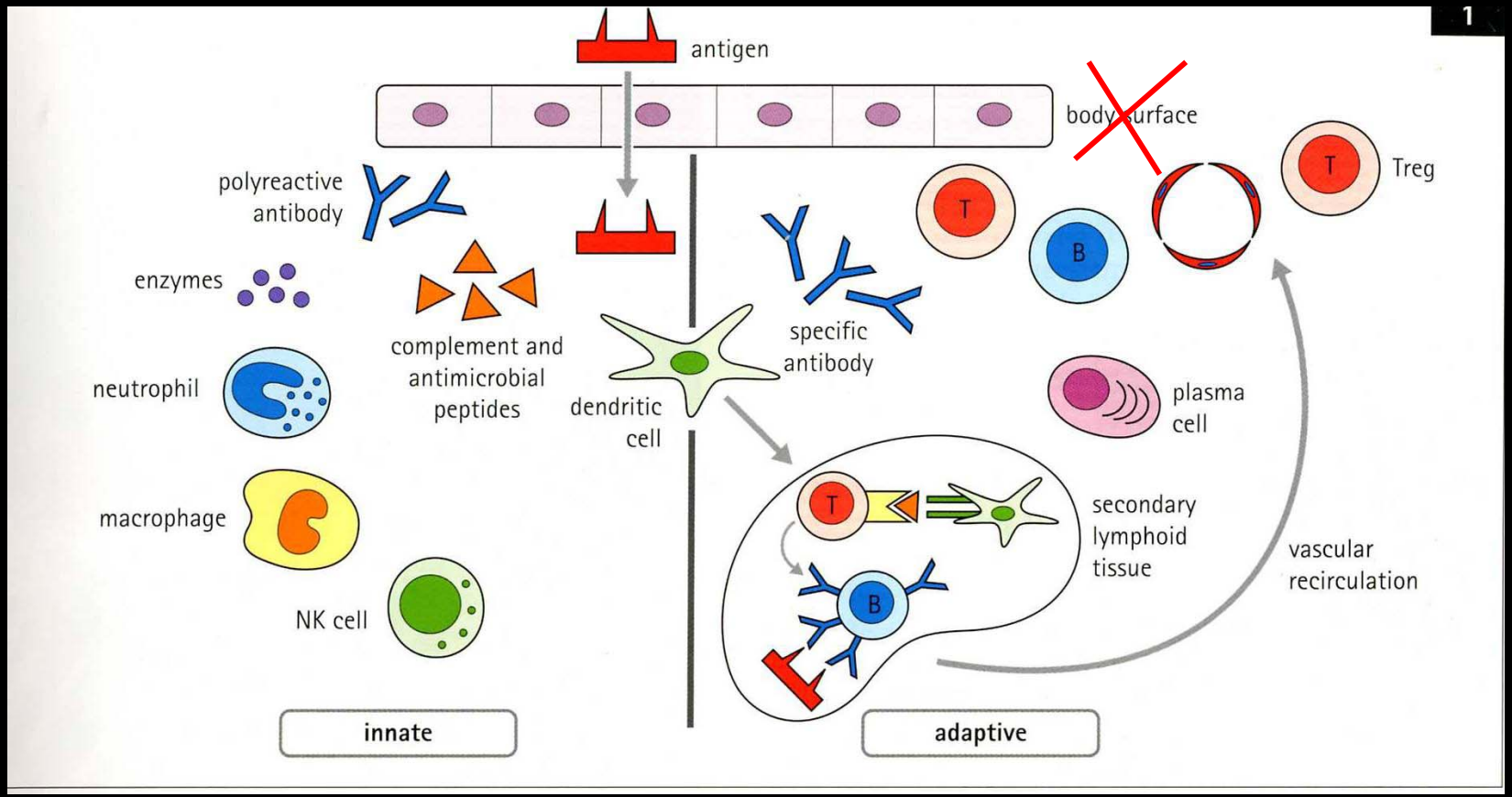

PL1
Length : 2787.91211 um

« The gastrointestinal tract is the largest immunologic organ in the body »

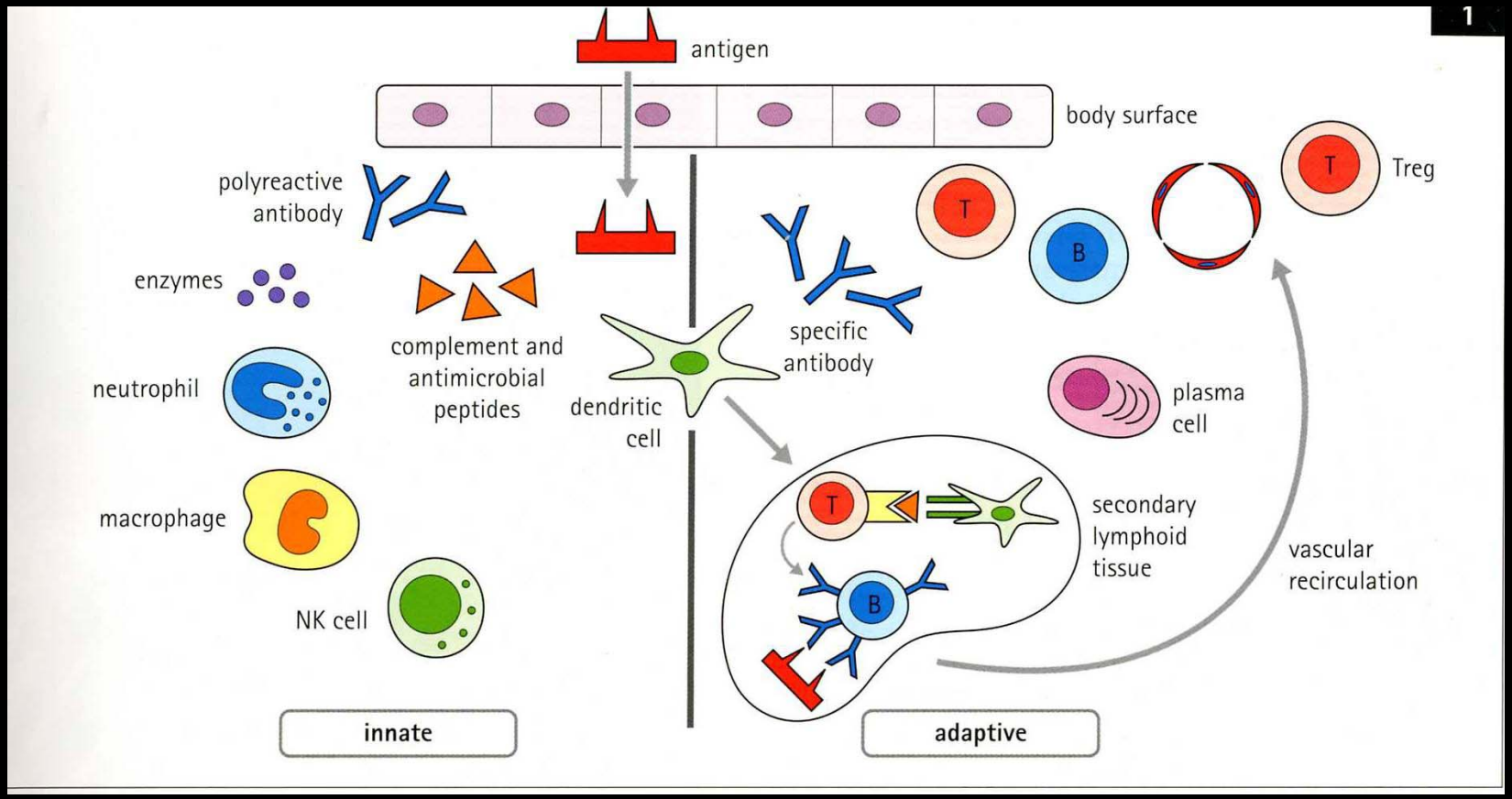
Chahine and Bahna, 2010

The intestinal ecosystem



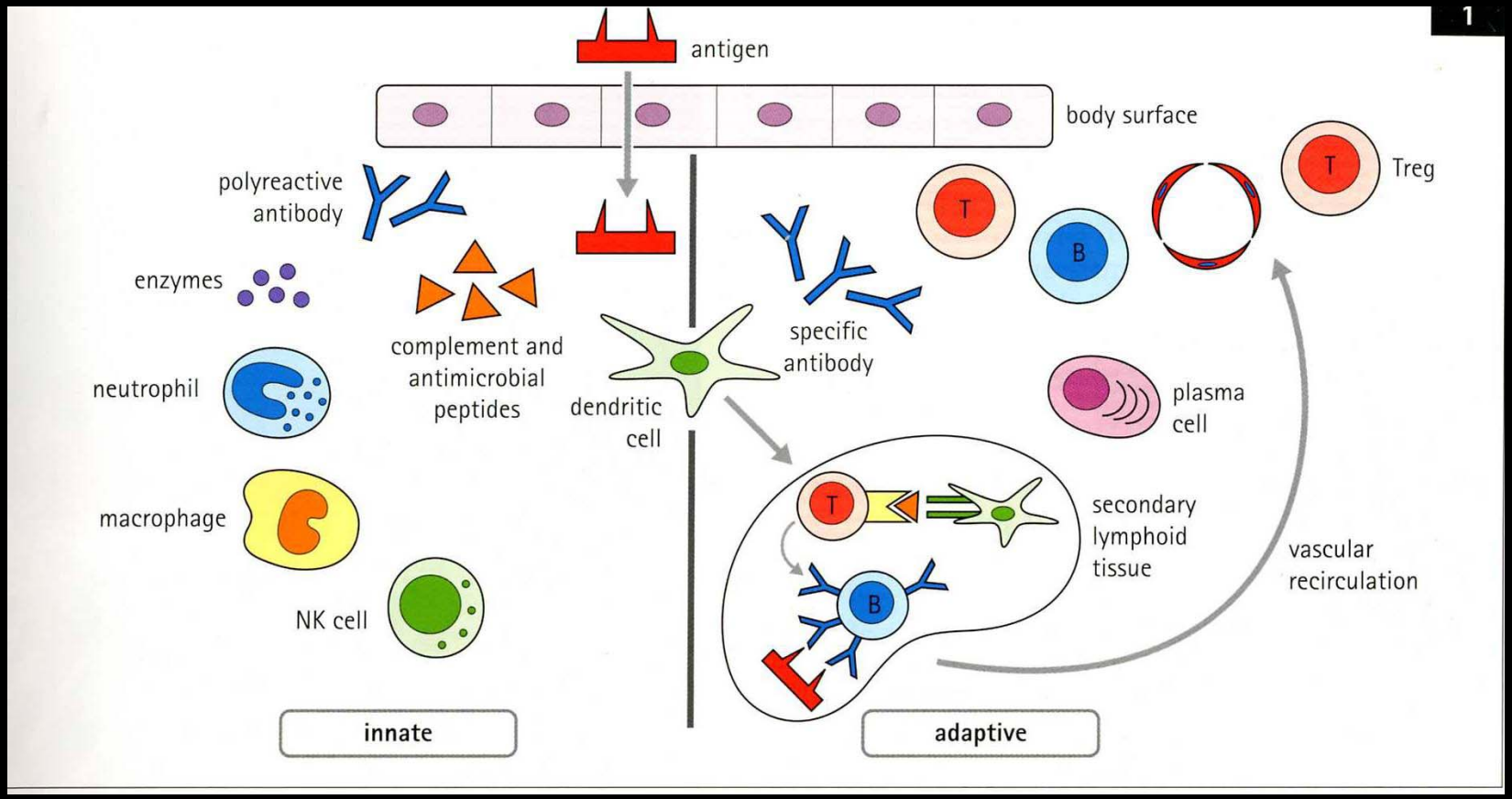


Day and Schulz, 2010



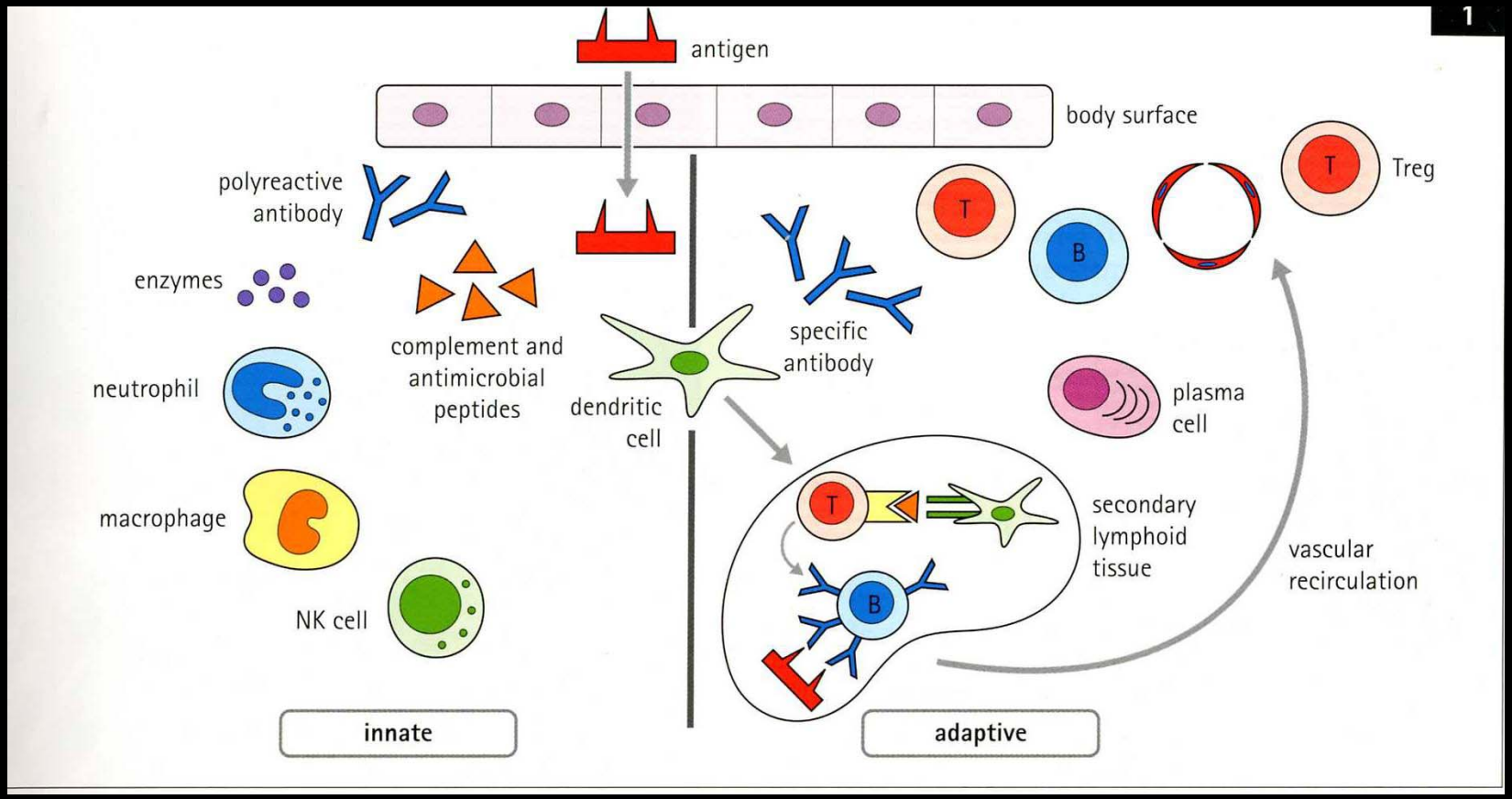
Day and Schulz, 2010

Antigens = immunogens = triggers of immune response = foreign proteins



Day and Schulz, 2010

Innate immune response = inflammation

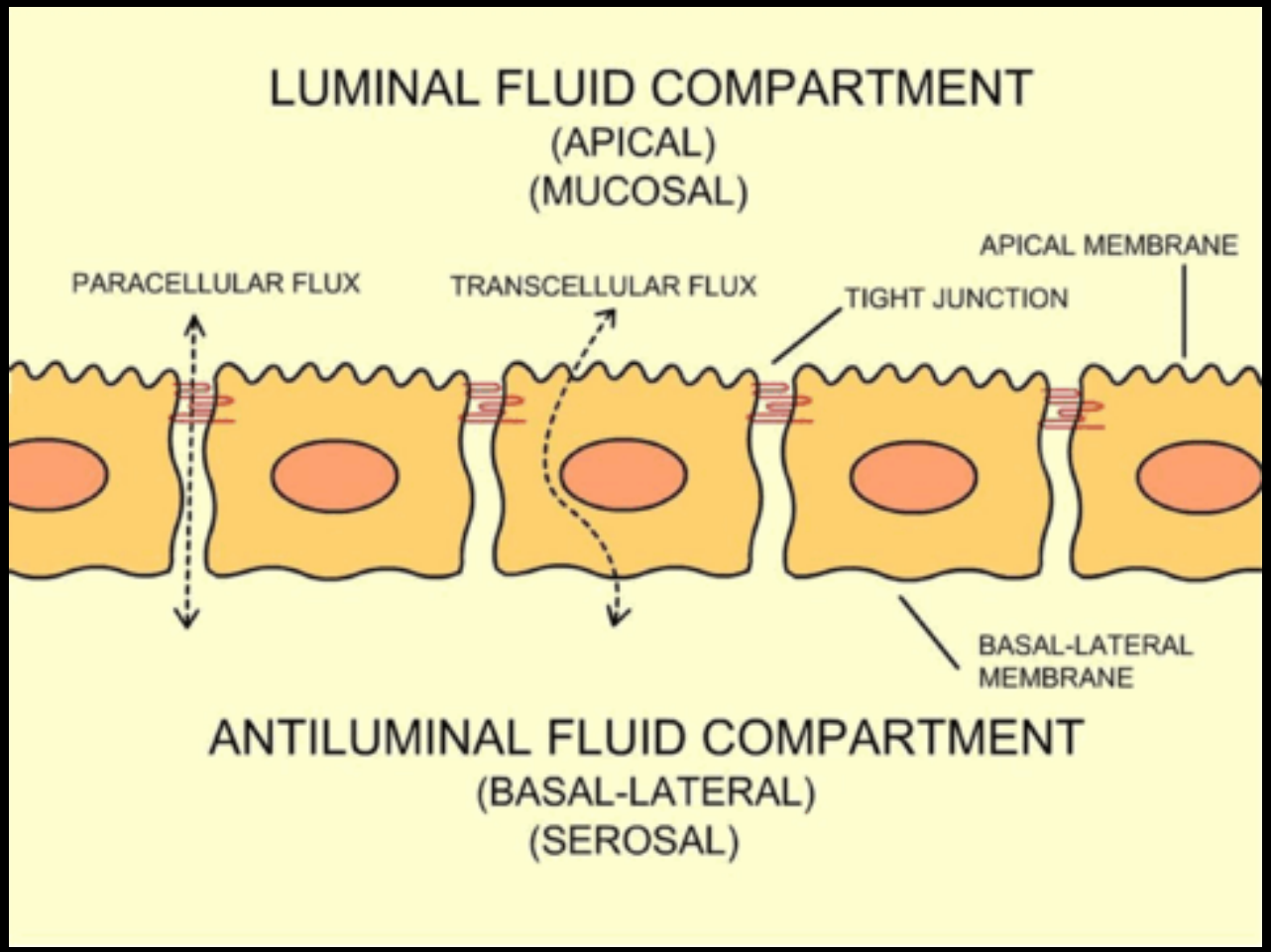


Day and Schulz, 2010

Innate immune response = inflammation

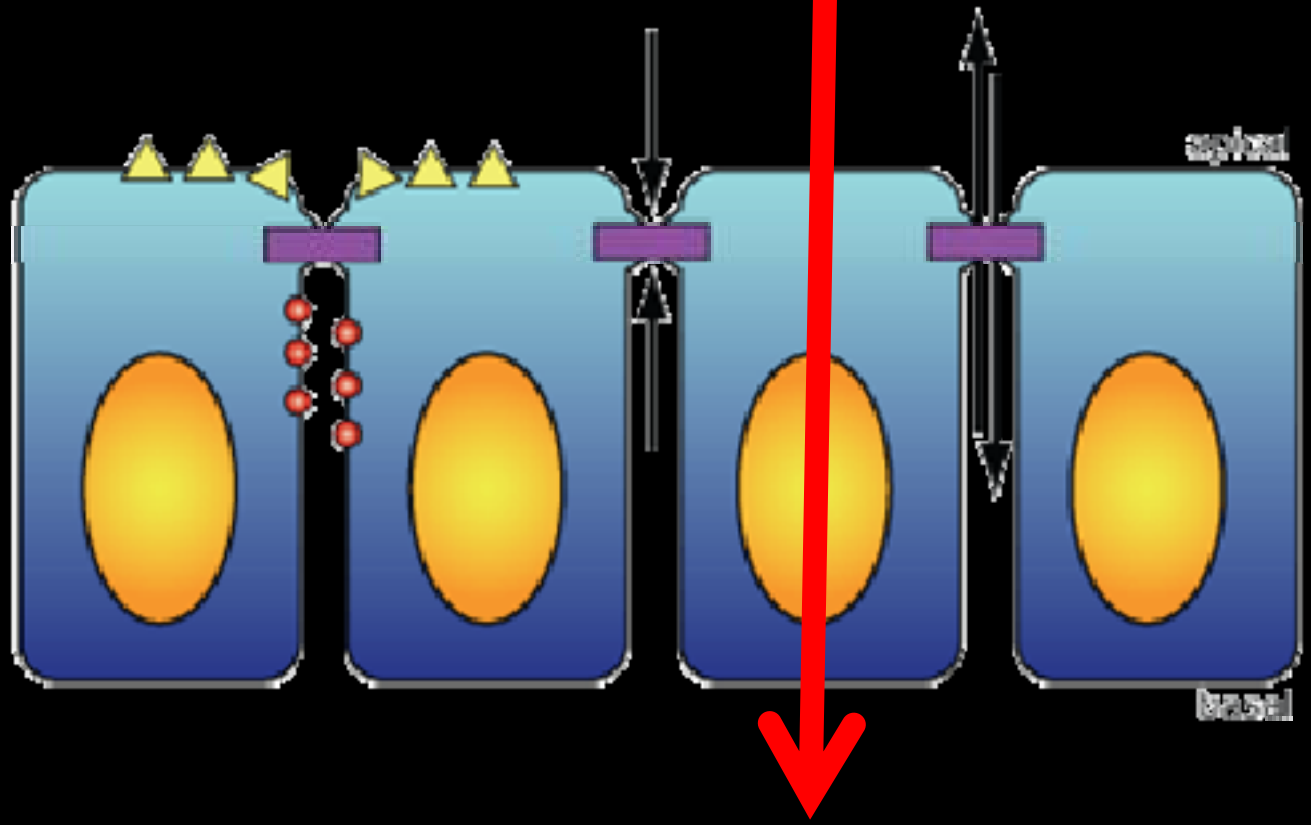
Inflammation = wet litter

The intestinal mucosal barrier



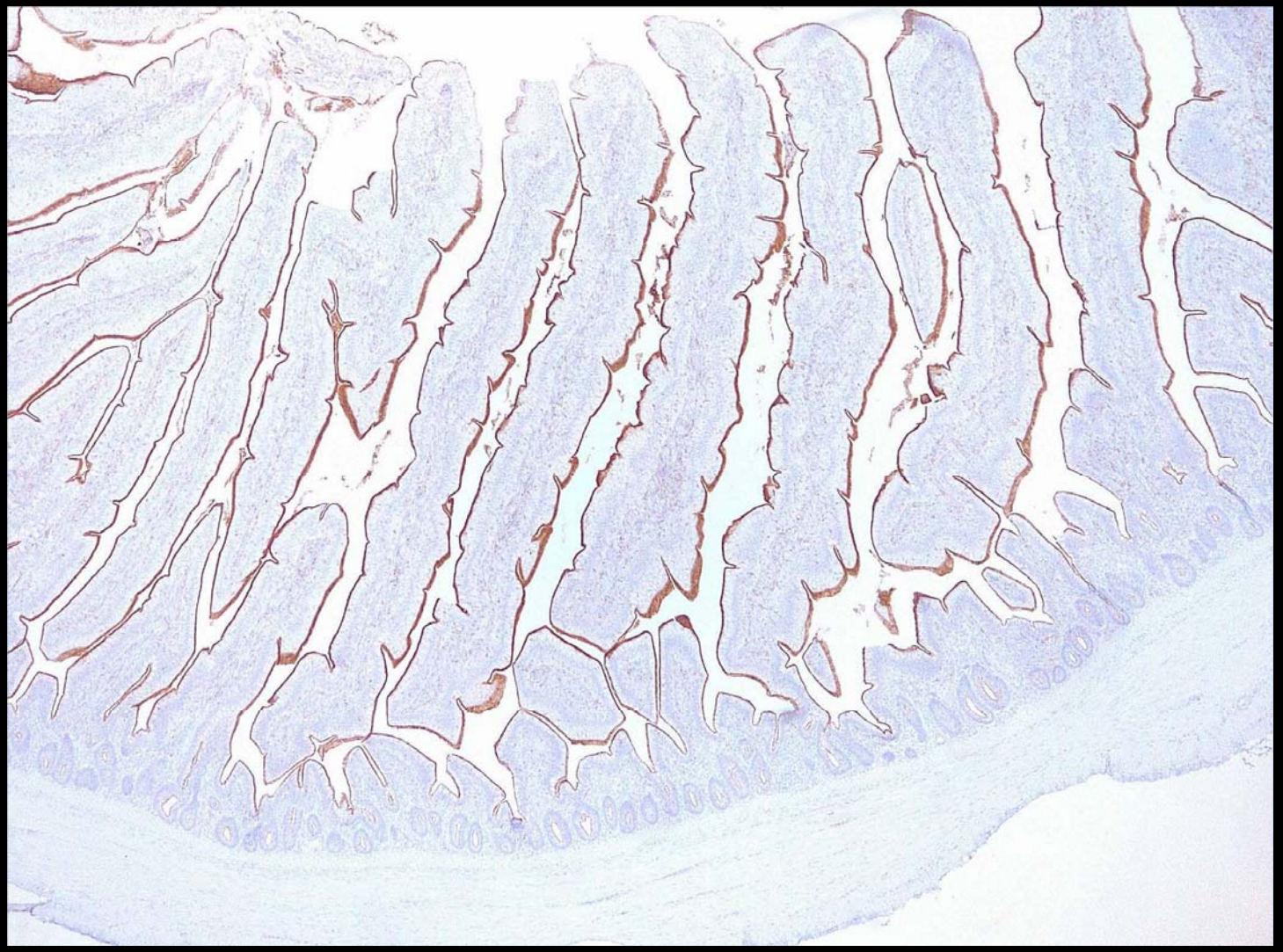
(Mullin et al., 2009)

Transcellular pathway



Mostly no immunological consequences, as processed by epithelial cell

P-gp, the MDR_1 gatekeeper of the transcellular pathway



C219 immunohistochemical stain of P-gp at the brush border of broiler jejunal epithelium

P-gp knock-out mice develop spontaneous intestinal inflammation under SPF conditions (Banner et al., 2004)

Nutritional factors affecting P-gp expression:

* Grapefruit

Nutritional factors affecting P-gp expression:

* Grapefruit

Polyphenols

Cardiovascular protection
Anticancerogenic
Antioxidative

Nutritional factors affecting P-gp expression:

* Grapefruit

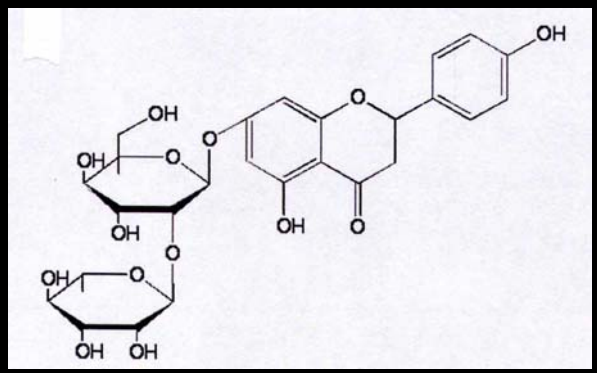
Polyphenols

↓

Flavonoids

↓

Naringin



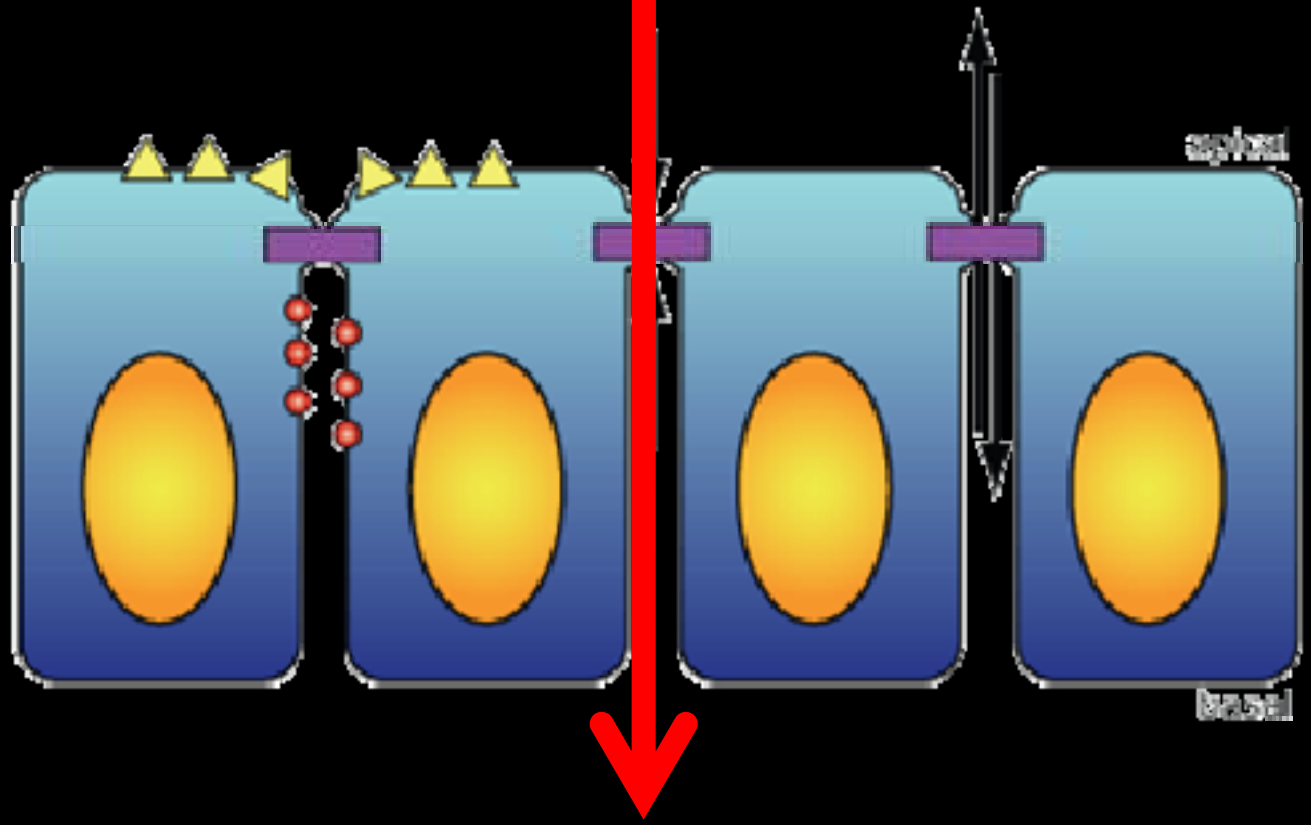
Nutritional factors affecting P-gp expression:

* Grapefruit

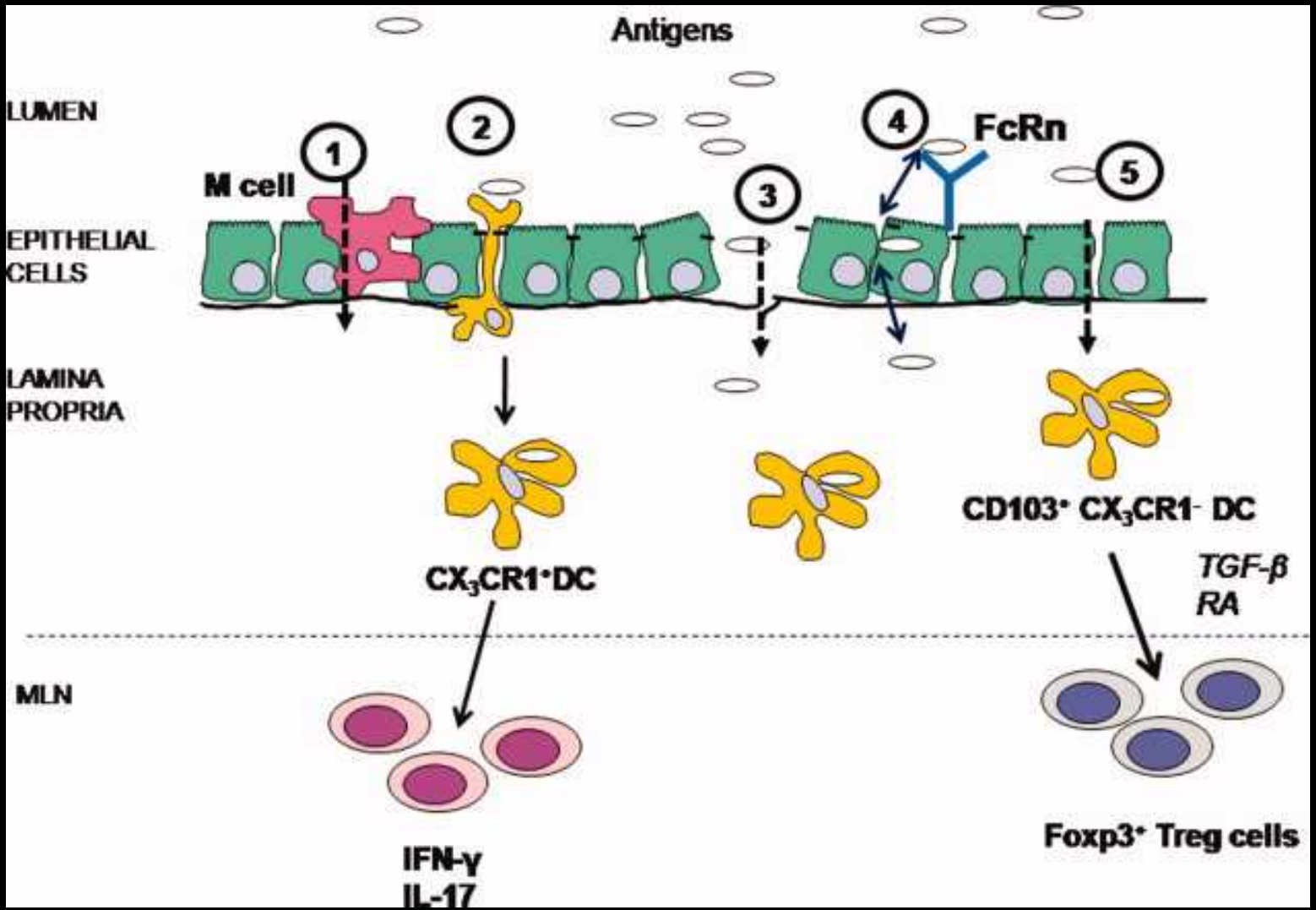
→ Concentration in citrus pulp?

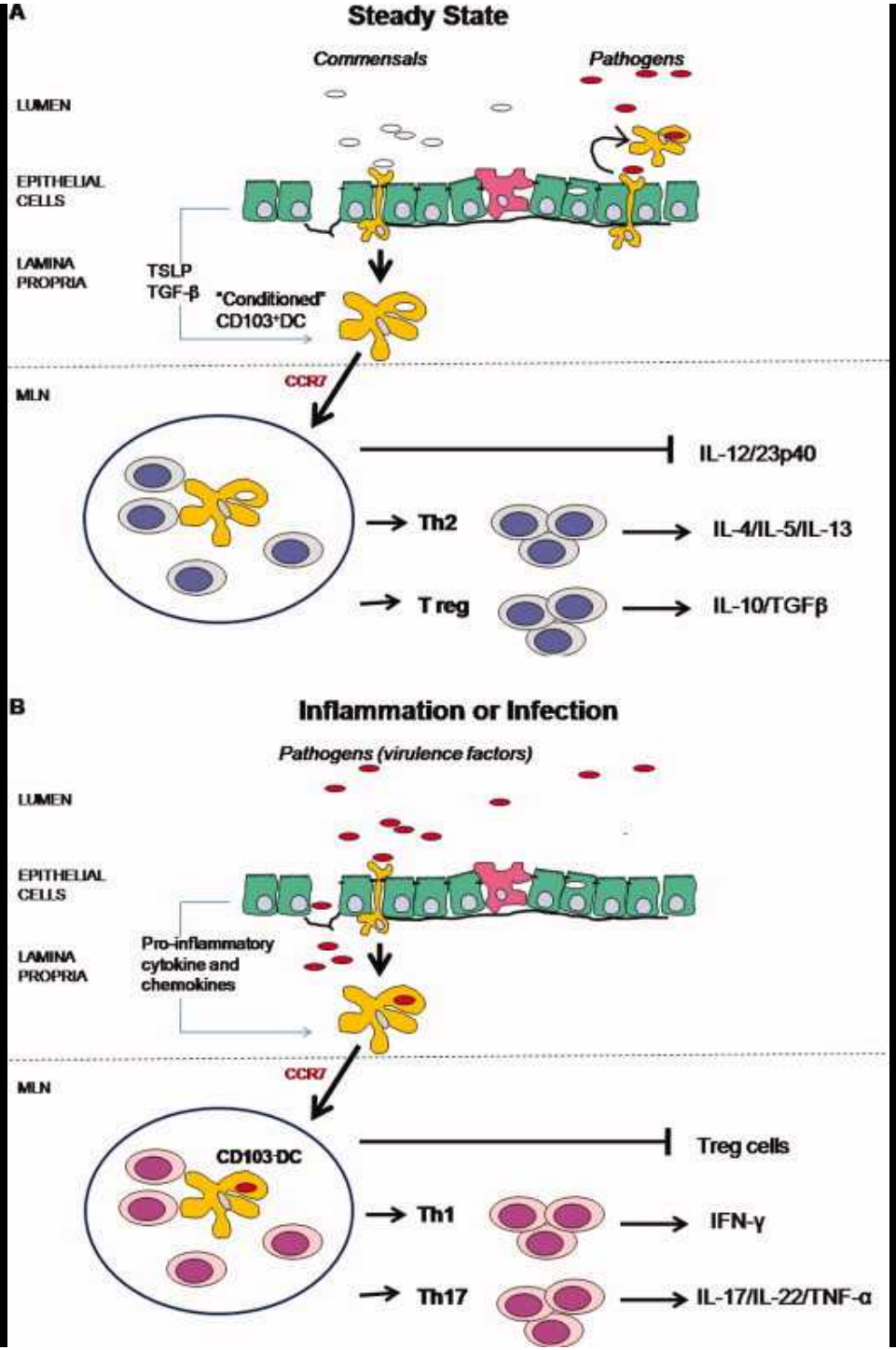
Species differences in the effect !

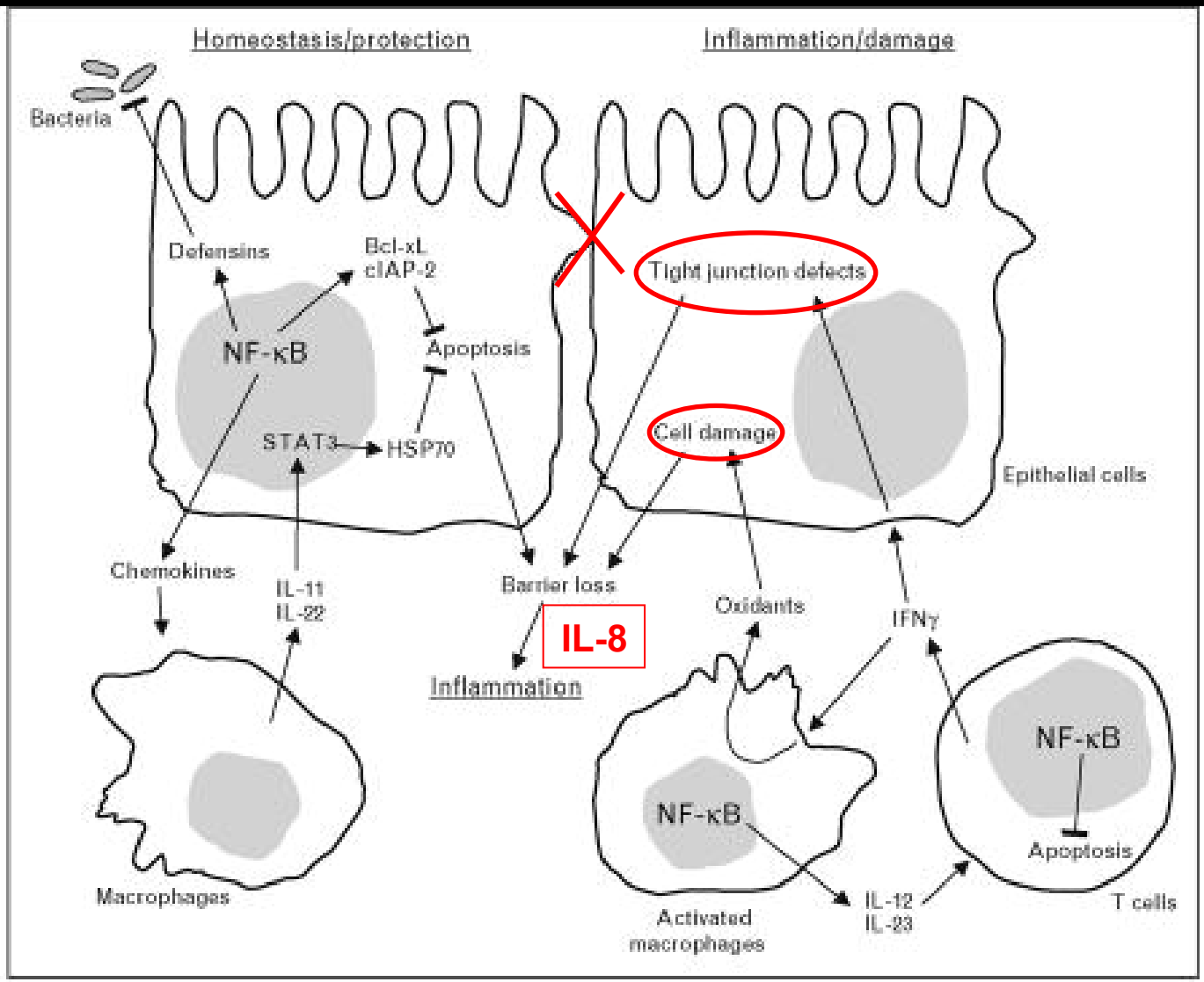
Paracellular pathway



Immunological consequences probable, as contact with basolateral surface

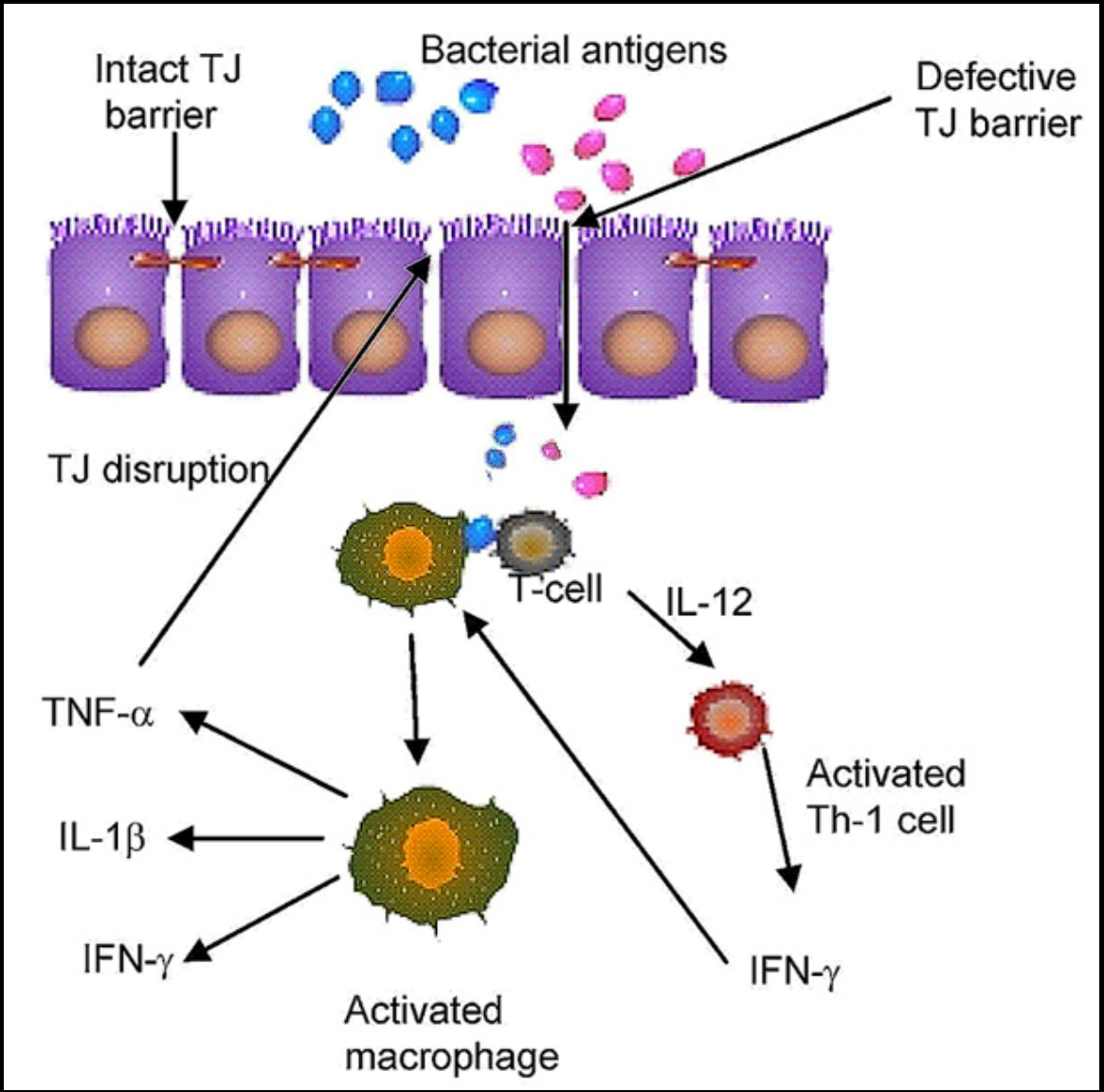

Antigens
LUMEN
M cell
EPITHELIAL CELLS
LAMINA PROPRIA
1
2
3
4
FcRn
5
CX3CR1+DC
CD103+ CX3CR1- DC
TGF-β
RA
MLN
IFN-γ
IL-17
Foxp3+ Treg cells


A
Steady State
Commensals
Pathogens
LUMEN
EPITHELIAL CELLS
LAMINA PROPRIA
TSLP
TGF-β
"Conditioned" CD103+DC
CCR7
MLN
IL-12/23p40
Th2
IL-4/IL-5/IL-13
T reg
IL-10/TGFβ
B
Inflammation or Infection
Pathogens (virulence factors)
LUMEN
EPITHELIAL CELLS
LAMINA PROPRIA
Pro-inflammatory cytokine and chemokines
CCR7
MLN
CD103-DC
Treg cells
Th1
IFN-γ
Th17
IL-17/IL-22/TNF-α



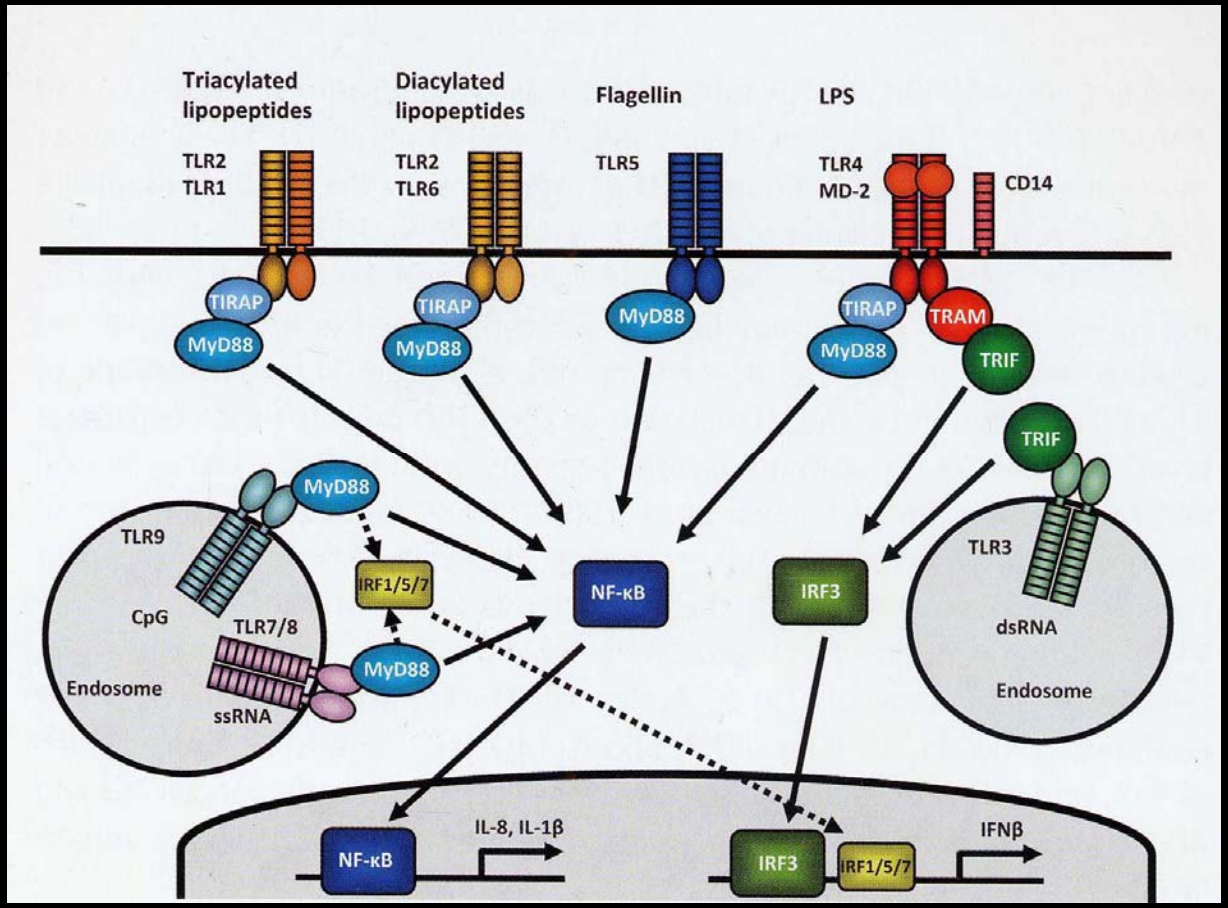
The scheme depicts a simplified overview of key intestinal functions of NF-κB under conditions of homeostasis and acute protection (left) or chronic inflammation and damage (right). NF-κB predominantly acts in epithelial protection in homeostasis and certain acute inflammatory events by inducing the transcription of antimicrobial and antiapoptotic products. The cytokine-inducing and antiapoptotic functions of NF-κB in macrophages and T cells are predominant under chronic inflammatory conditions. HSP, heat shock protein; NF-κB, nuclear factor-kappa B.

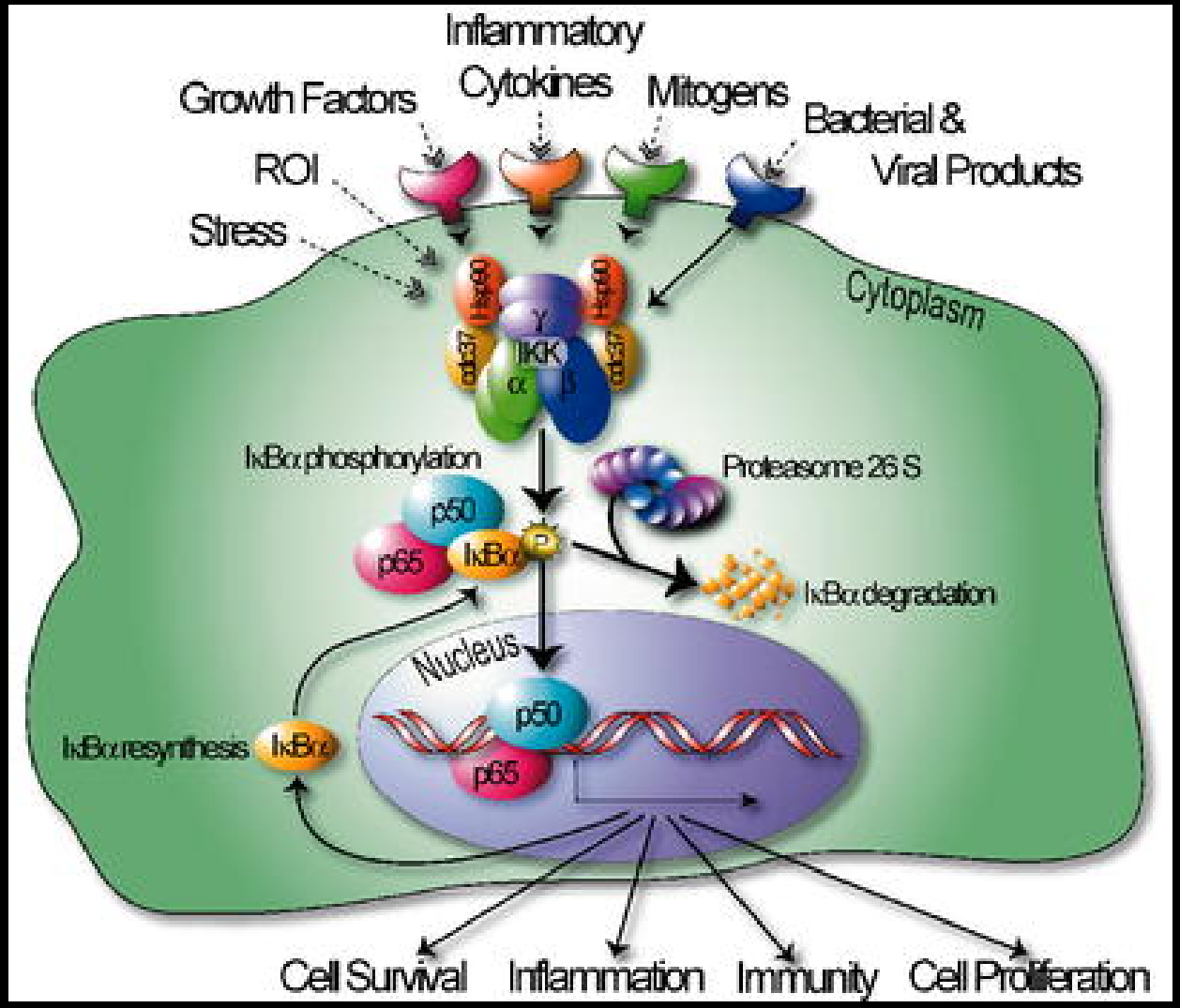
The vicious circle of tight junction barrier disruption



(Al-Sadi et al., 2009)

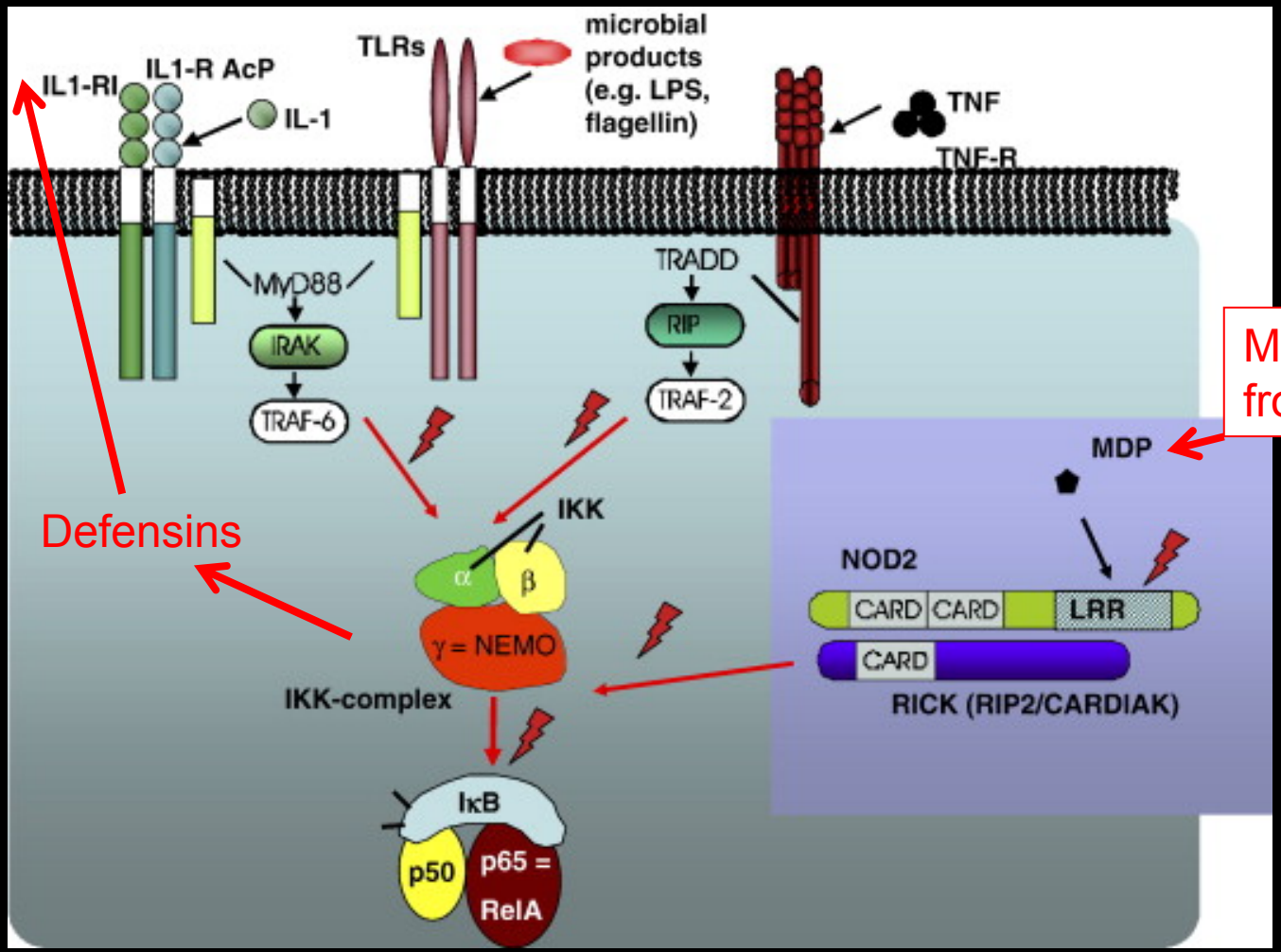
MAMPs and PRRs: triggers of the innate (inflammatory) immune response





IL-8

(Kumar et al., 2004)


IL1-RI
IL1-R AcP
IL-1
TLRs
microbial products (e.g. LPS, flagellin)
TNF
TNF-R
MyD88
IRAK
TRAF-6
TRADD
RIP
TRAF-2
MDP
Muramyl dipeptide from peptidoglycan
Defensins
IKK
α
β
γ = NEMO
IKK-complex
NOD2
CARD
CARD
LRR
CARD
RICK (RIP2/CARDIAK)
IκB
p50
p65 = RelA

Feed formulation affecting intestinal health

Experimental set up

– 2 ≠ carbohydrate sources

Corn

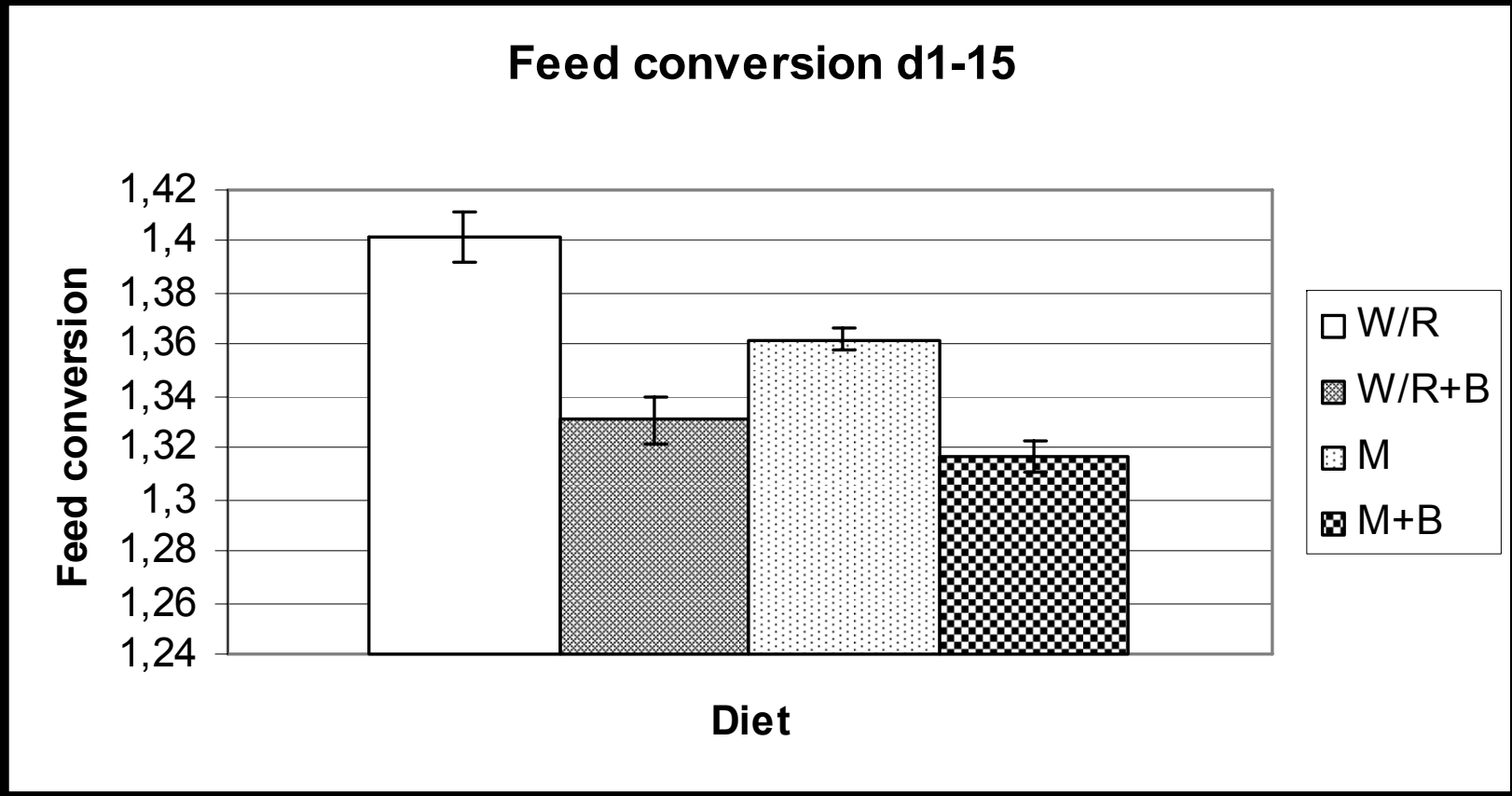
↔

Wheat/Rye

different only in NSP content

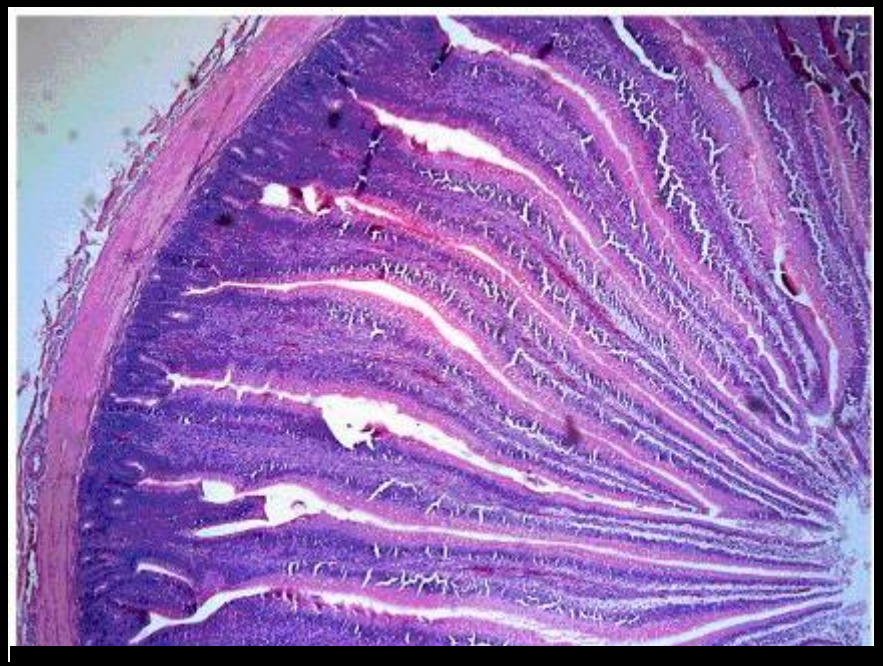
Results

Feed conversion

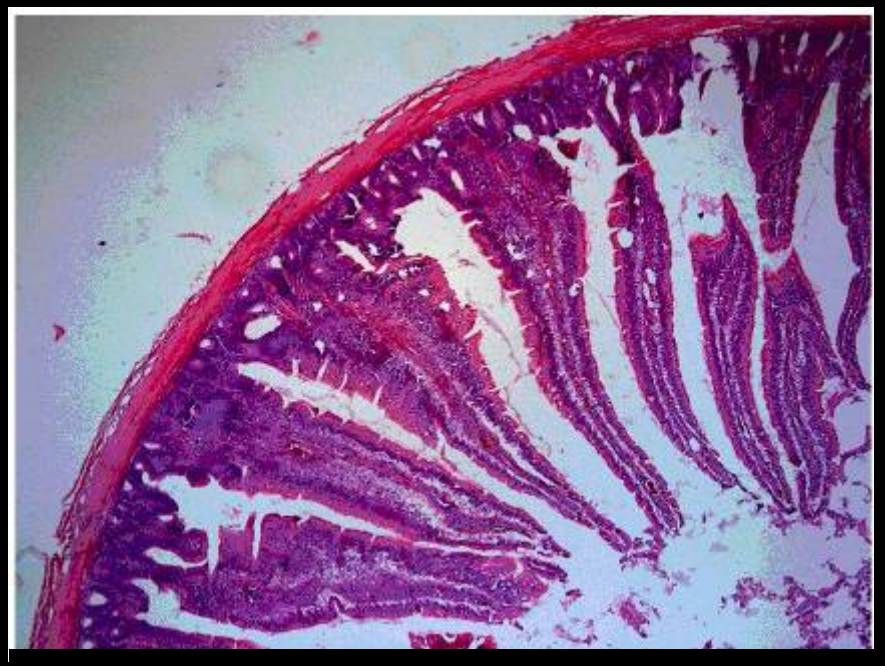


Results

Villus length



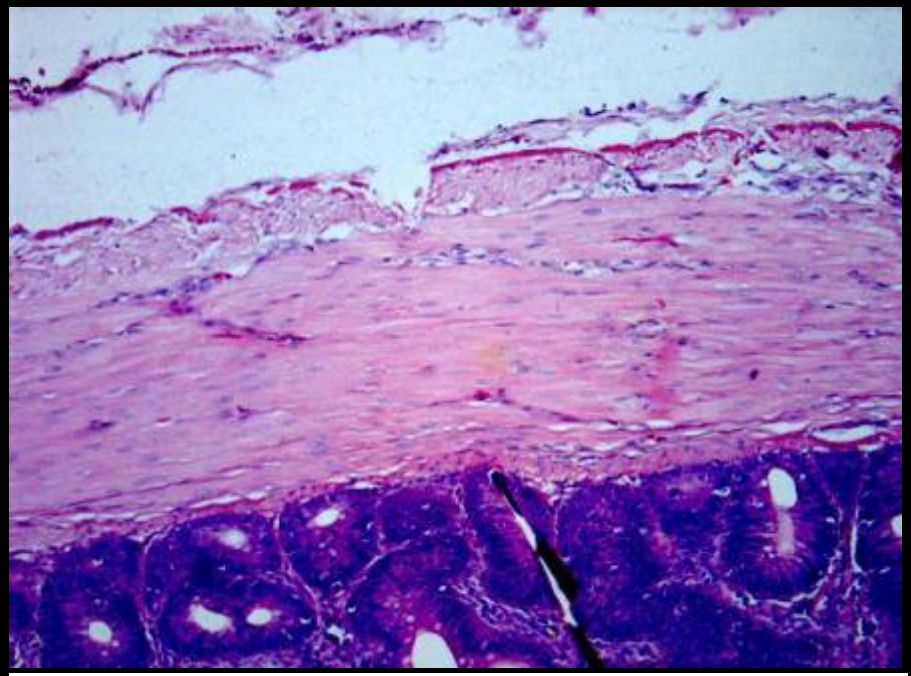
Villi Duodenum M+B diet (dag 15)



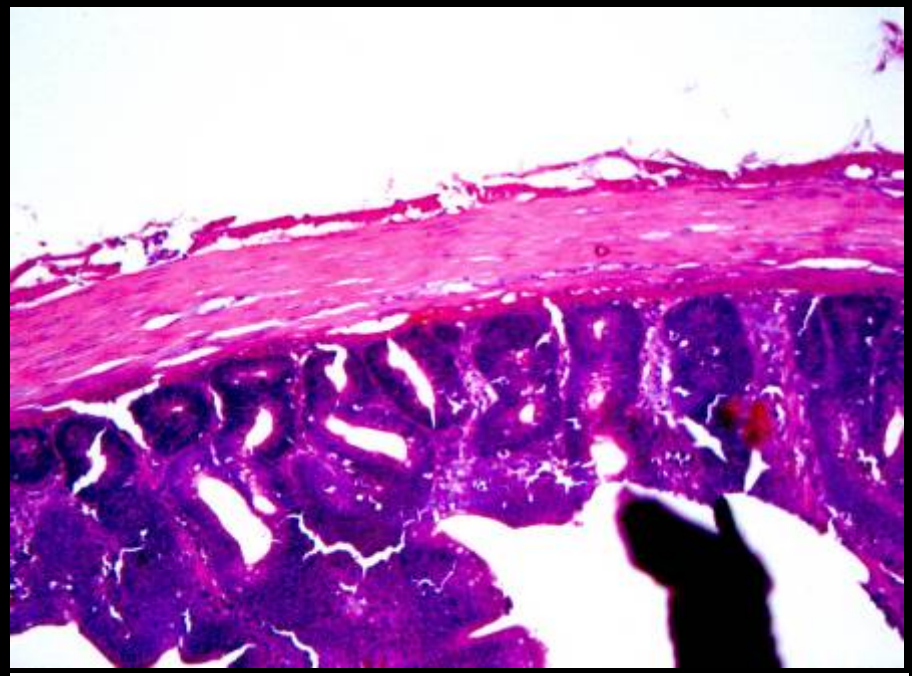
Villi Duodenum W/R diet (dag 15)

Results

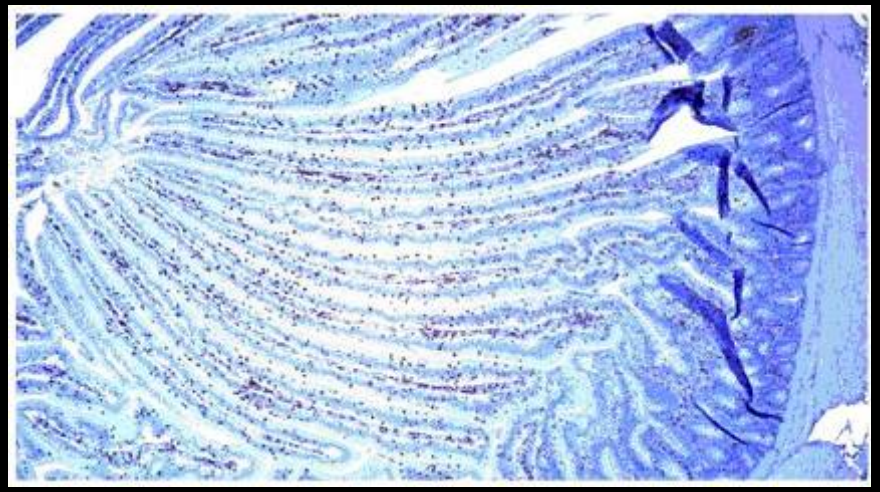
Muscularis thickness



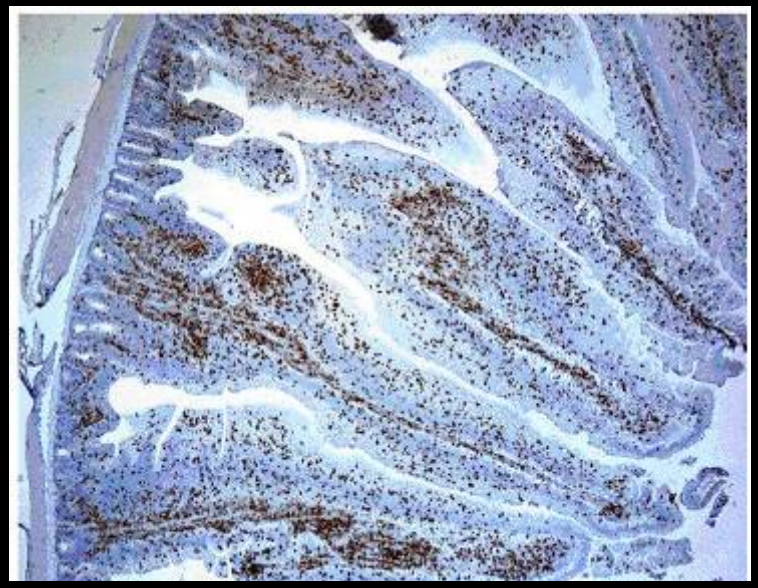
M+B: Thickness tunica muscularis duodenum (day 15) (20x)



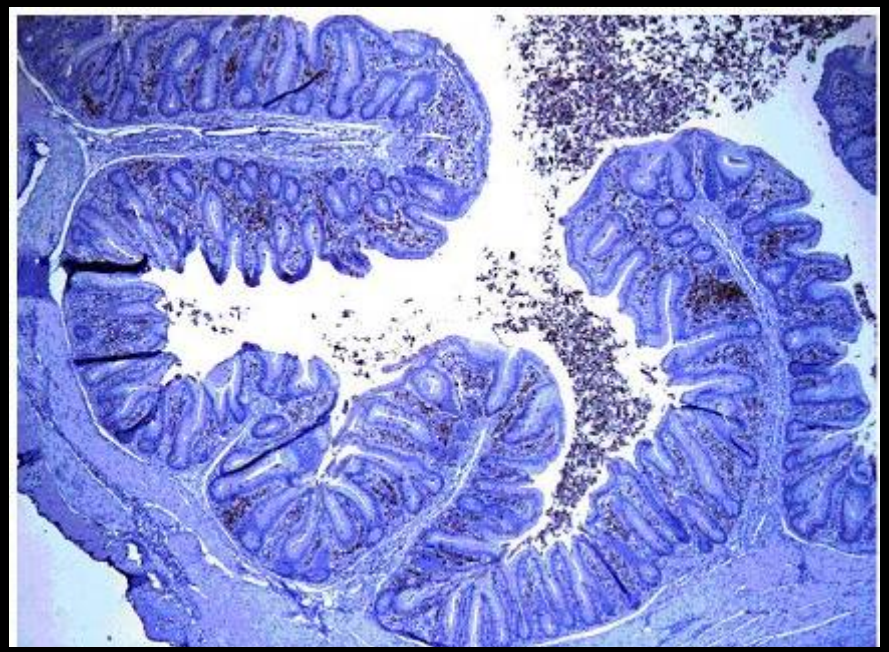
W/R: Thickness tunica muscularis duodenum (day 15) (20x)



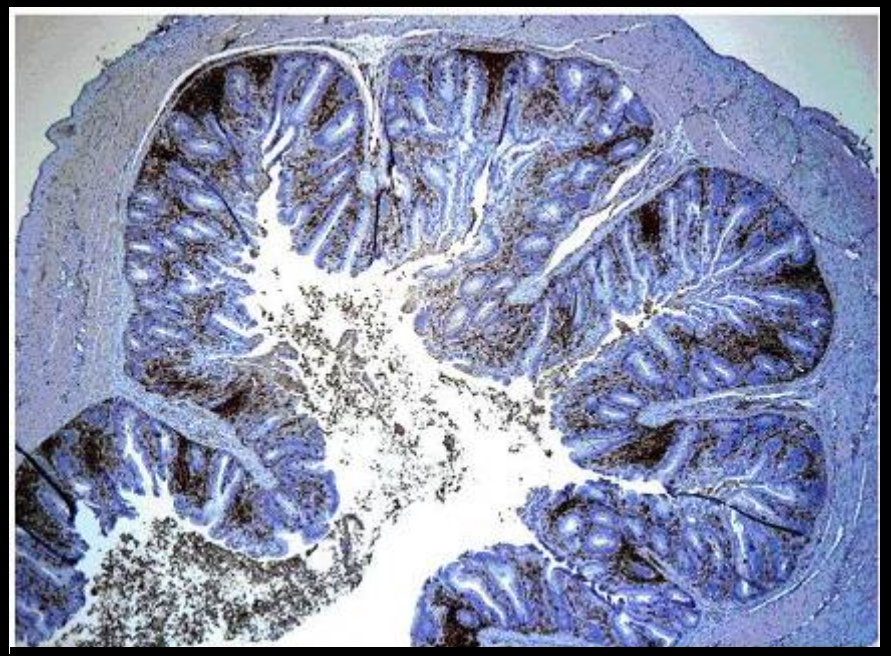
M+B: T-cel infiltration in duodenum



W/R: T-cel infiltration in duodenum



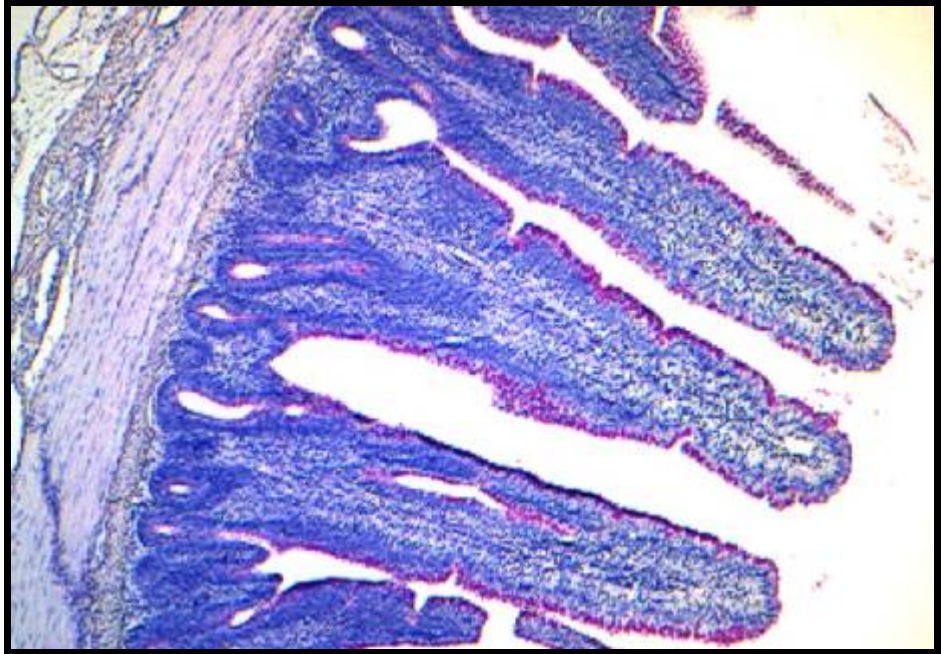
M+B: T-cel infiltration in caecum



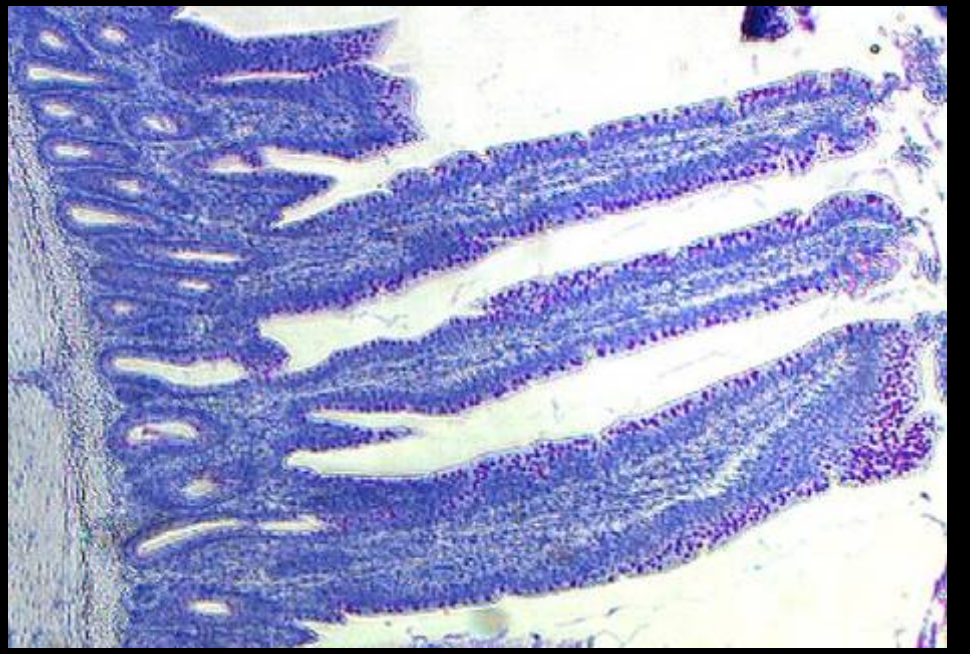
W/R: T-cel infiltration in caecum

Results

Goblet cells



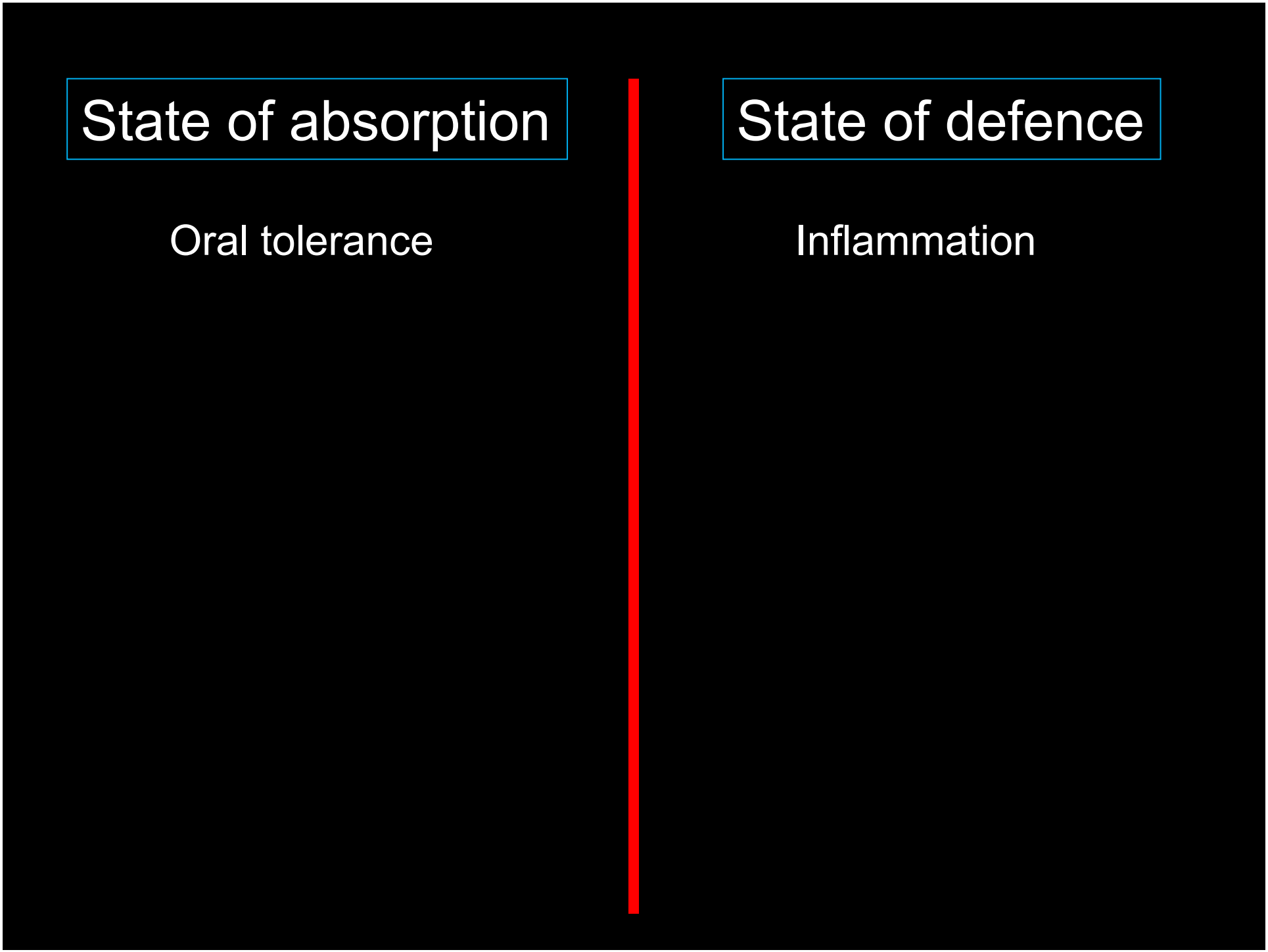
W/R: ileum (100 x)



M: ileum (100x)

State of absorption

State of defence


State of absorption
State of defence
Oral tolerance
Inflammation

State of absorption

Oral tolerance
Strong tight junctions

State of defence

Inflammation
« leaky gut »

State of absorption

Oral tolerance
Strong tight junctions
Long slender villi

State of defence

Inflammation
« leaky gut »
Short thick villi

State of absorption

Oral tolerance
Strong tight junctions
Long slender villi
Few T-lymphocytes

State of defence

Inflammation
« leaky gut »
Short thick villi
Massive T-lymphocytes

Table 1 Main enteric pathogens and /or toxins that modify epithelial TJs and mechanisms used by these pathogens at the TJ level

Enteric Pathogens	*Mechanisms*
S. typhimurium *E. coli* (EPEC, EHEC,DAEC) *S. flexneri* *H. pylori* *Y. pseudotuberculosis*	Inducing neutrophil transepithelial migration
C. difficile (toxins A and B) *C. botulinum* (toxins C2 and C3) *C. sordelli* *C. perfringens* (toxin CPE) *L. monocytogenes* *V. cholerae* (toxins RTX and ZOT) *B. fragilis* (toxin bacteroides) *E. coli* (toxin CNF-1)	Remodeling of actin cytoskeleton
E. coli (EPEC, EHEC) *S. flexneri* *H. pylori* (toxin Vac A)	Activation of cellular signal transduction
L. monocytogenes *B. fragilis* *C. difficile* (toxins A) *C. perfringens* (enterotoxin) *S. flexneri* *E. coli* (DAEC)	Modification of TJ proteins

(Hofman, 2003)

Nutritional factors affecting tight junctions:

- Zinc (and ochratoxin A) (Ranaldi et al., 2009)
- Glutamine (alanyl-glutamine) (Furst et al., 2004)
- PUFA (Willemsen et al., 2008)
- Polyphenols – flavonoids (quercetin) (Amasheh et al., 2009)
- Butyrate (depending on concentration)

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- Polyphenols – flavonoids (quercetin) (Amasheh et al., 2009)
- Butyrate (depending on concentration)
- NSP?

The normal intestinal barrier allows small amounts of antigens to pass through the mucosa to interact with the immune system

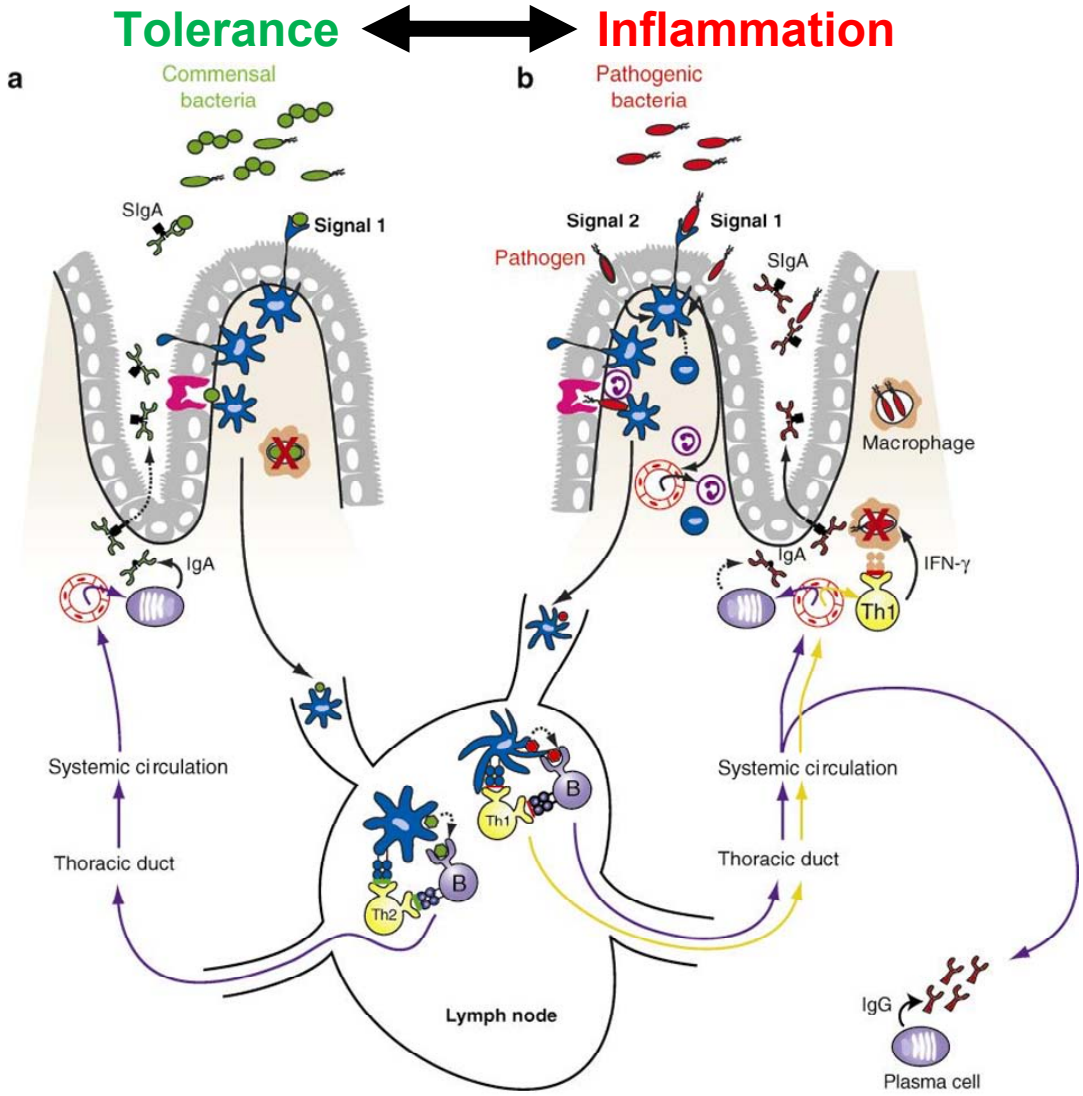
(Keita and Söderholm, 2010)

Small amounts of intact food proteins are absorbed by the gut, Amounting to a daily uptake of 130 – 190g of food protein

(Brandtzaeg, 2010)

Resulting in a sustained state of physiologic intestinal inflammation

(Chahine and Bahna, 2010)



Iwasaki A. 2007.
Annu. Rev. Immunol. 25:381–418

Annual Reviews

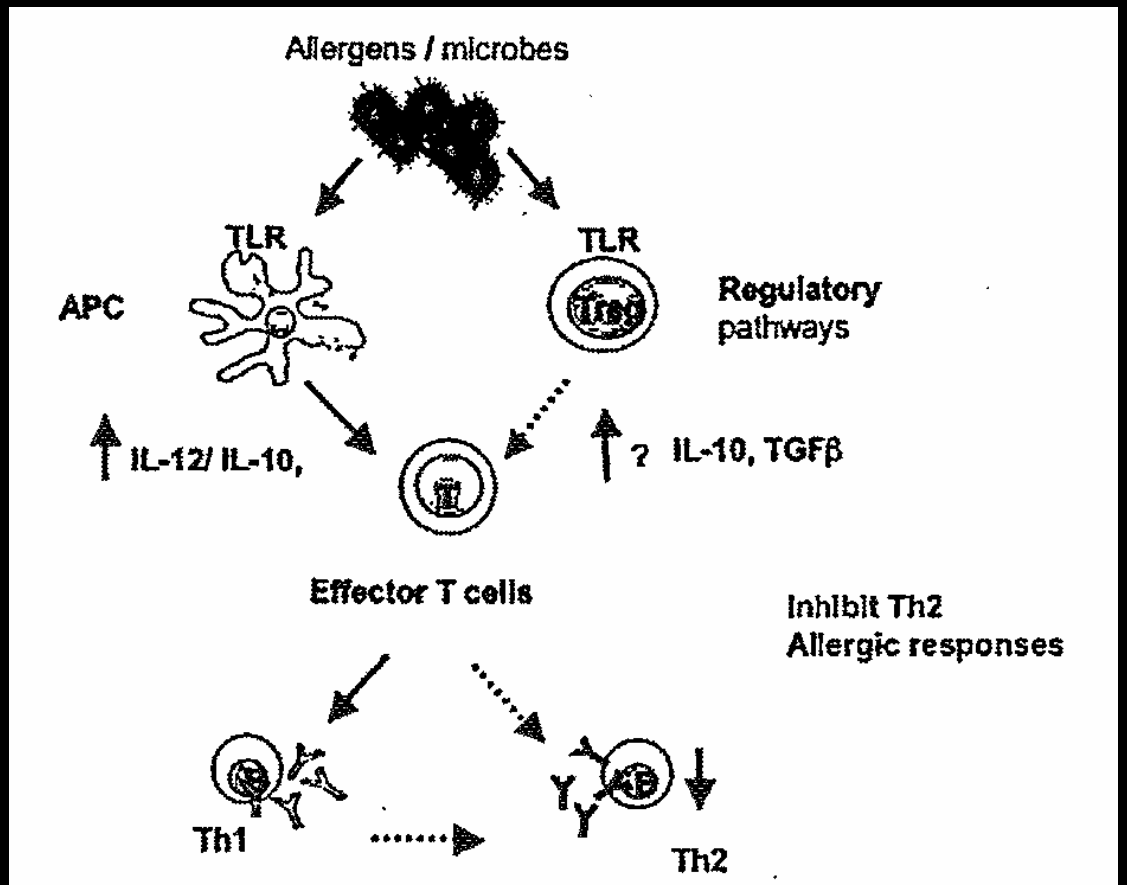
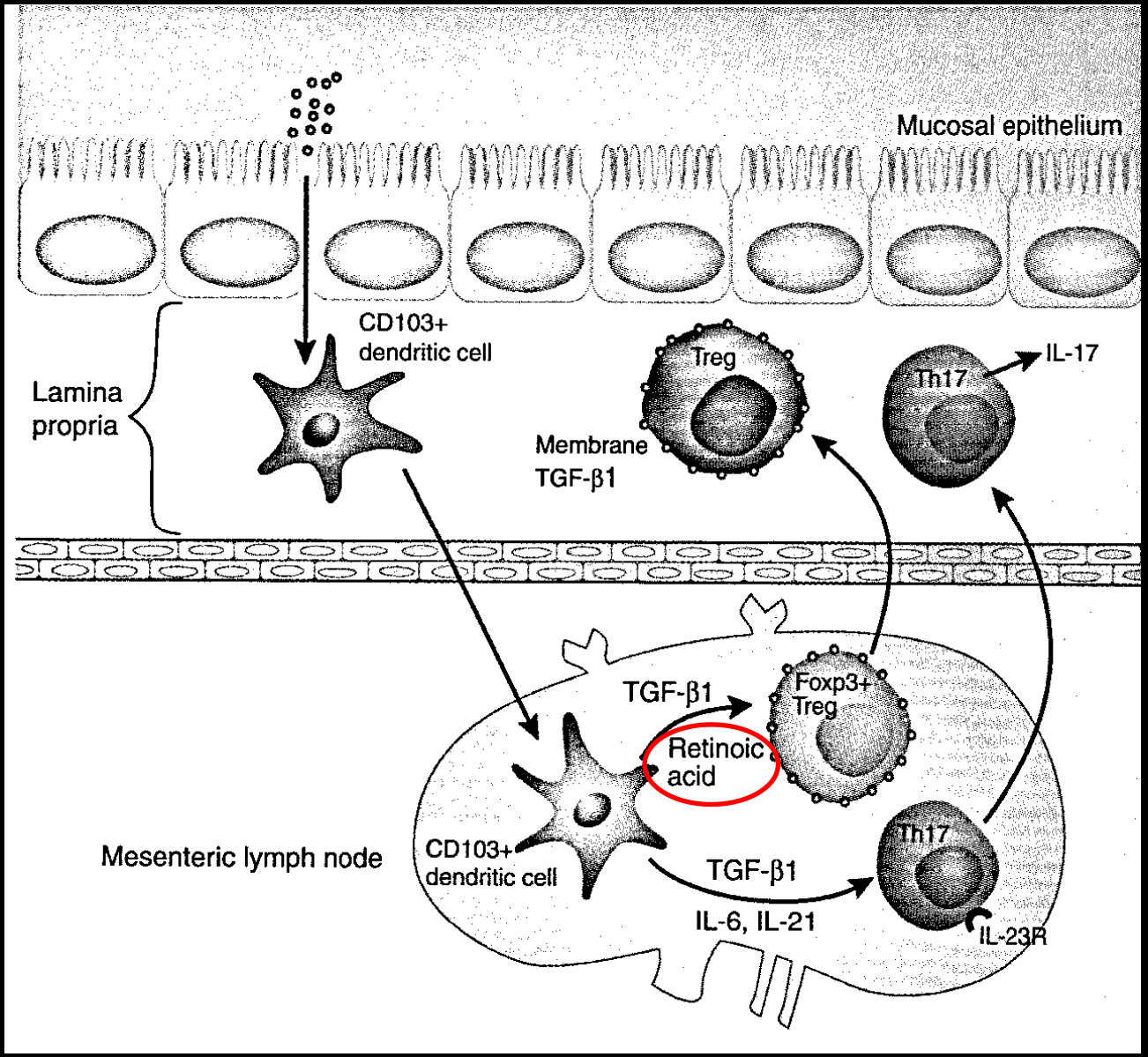


Fig. 1. Immune pathways involved in producing tolerance (TLR – toll-like receptors, APC – antigen-presenting cells, Treg – regulatory T cell, IL – interleukin, TGF – tumour growth factor, Th – T helper).

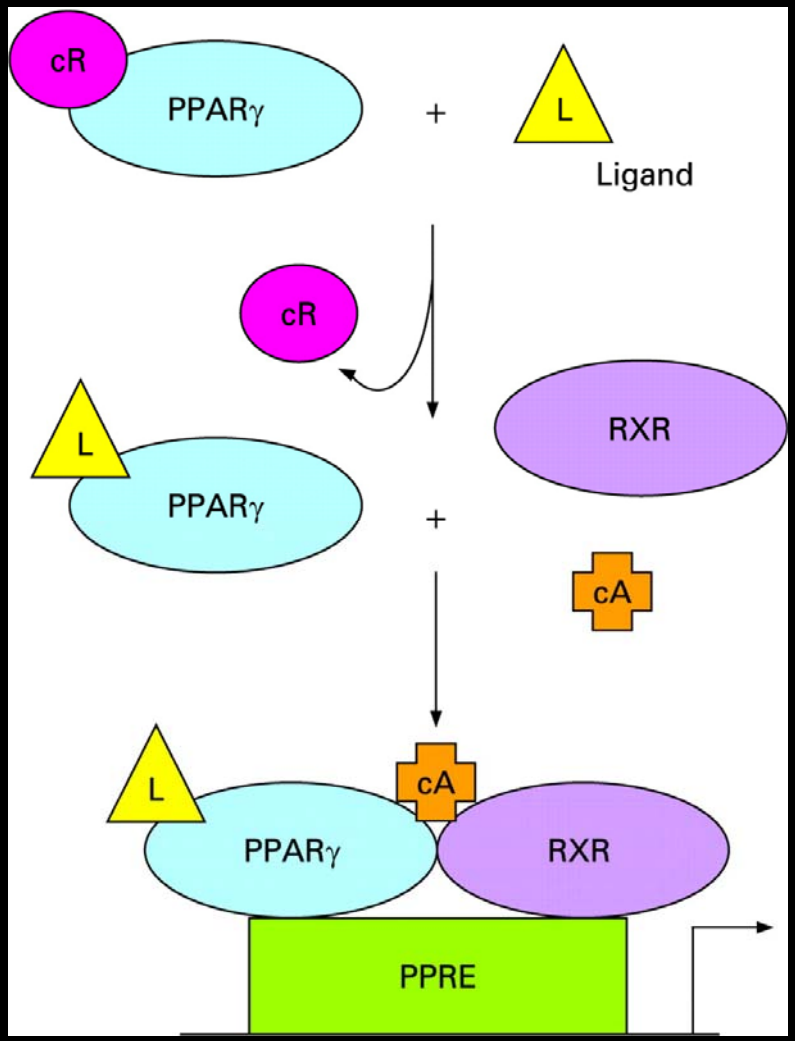
(Prescott, 2008)

Vitamin A is required for oral tolerance



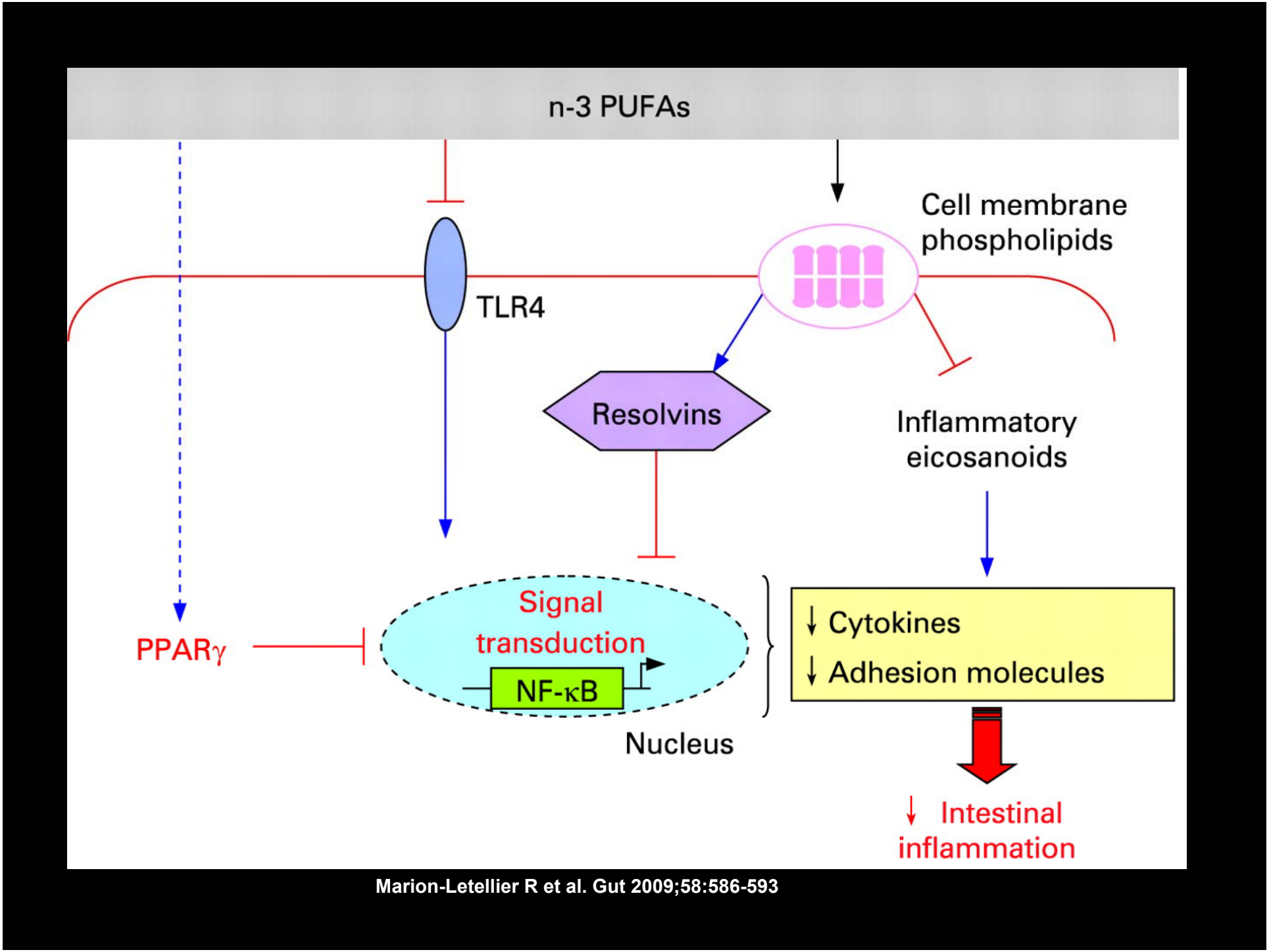
(Strober, 2008)

Peroxisome proliferator-activated receptor gamma (PPARγ) interacts with nuclear proteins that act as co-repressors (cR) and co-activators (cA).



Marion-Letellier R et al. Gut 2009;58:586-593

GUT



Marion-Letellier R et al. Gut 2009;58:586-593

Table 1 Demonstrated anti-inflammatory effects of nutrients through peroxisome proliferator-activated receptor gamma (PPARγ) in intestine

Nutrient	Models
α-Linolenic acid, docosahexaenoic acid	Intestinal epithelial cells[29]
Eicosapentaenoic acid	Intestinal epithelial cells[29]
Conjugated linoleic acid	Intestinal epithelial cells,[30 38 40] dextran sodium sulfate colitis model[5 42]
15-Deoxy-Δ12,14-prostaglandin J2	Intestinal epithelial cells,[6 48] colitis,[6 49] immobilisation-induced stress model[50]
15-Hydroxyeicosatetraenoic acid, 13-Hydroxyoctadecadienoic acid, 13-Oxooctadecadienoic acid,	Intestinal epithelial cells[44 45]
Butyrate	Intestinal epithelial cells[24–26]
Glutamine	Ischaemia–reperfusion rats[54]
Curcumin	TNBS-induced colitis,[59 60 62] sepsis[63]
Capsaicin	Intestinal epithelial cells[65]
Resveratrol	Intestinal epithelial cells[71]
Vitamin E	Intestinal epithelial cells[72]

TNBS, 2,4,6-trinitrobenzene sulfonic acid.

Marion-Letellier et al., 2009

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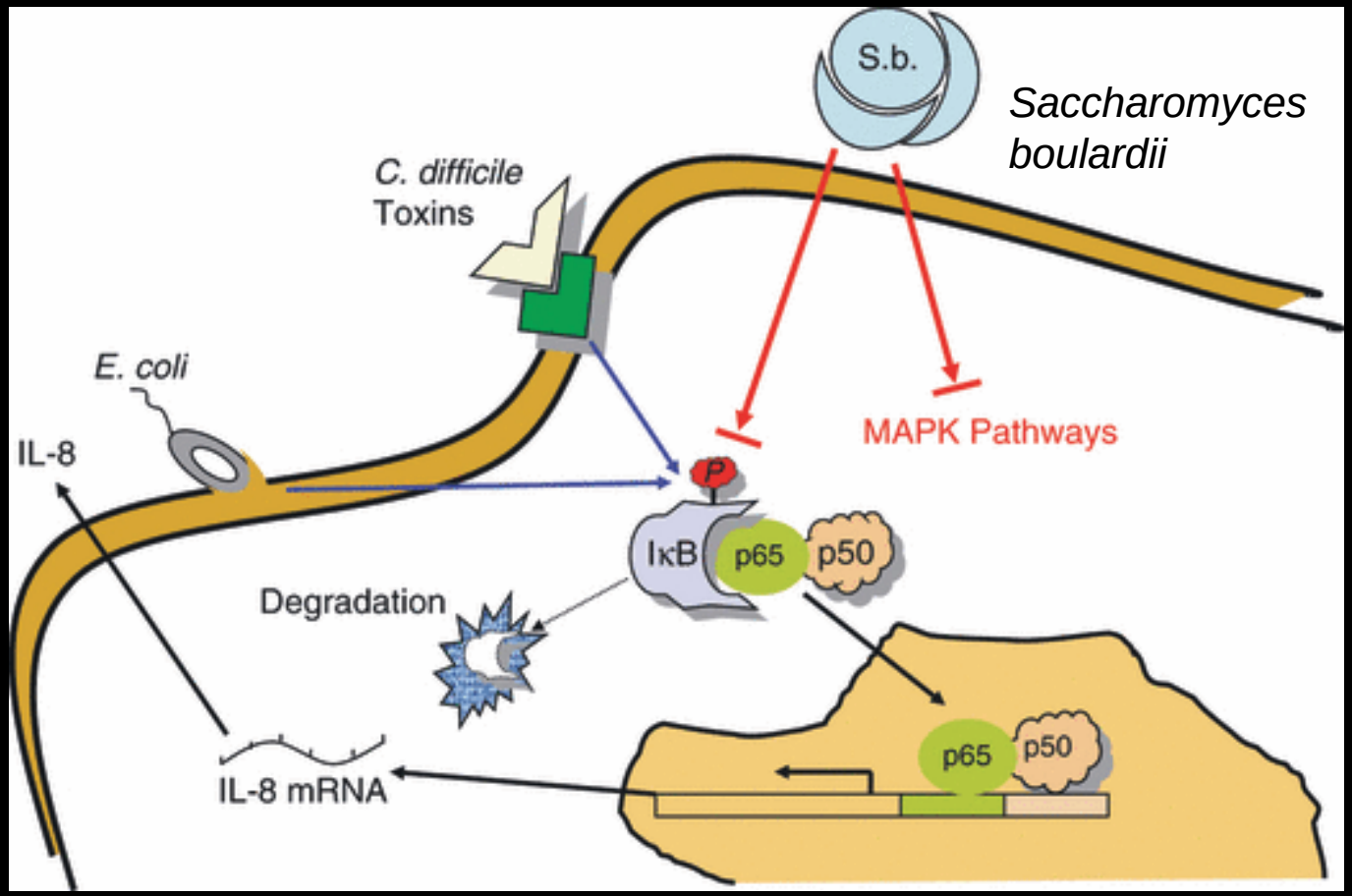
TNBS, 2,4,6-trinitrobenzene sulfonic acid.

Saccharomyces boulardii **TNBS induced colitis**

Marion-Letellier et al., 2009

β-1,3/1,6-glucans



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Short communication

Oral administration of beta-1,3/1,6-glucan Macrogard® fails to enhance the mucosal immune response following oral F4 fimbrial immunisation in gnotobiotic pigs

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ABSTRACT

In this study gnotobiotic animals were used to see if the F4 fimbrial antigen of F4ac+ *Escherichia coli* is as immunogenic as in conventional pigs. In addition, the adjuvant effect

What happens when tolerance mechanisms fail ?

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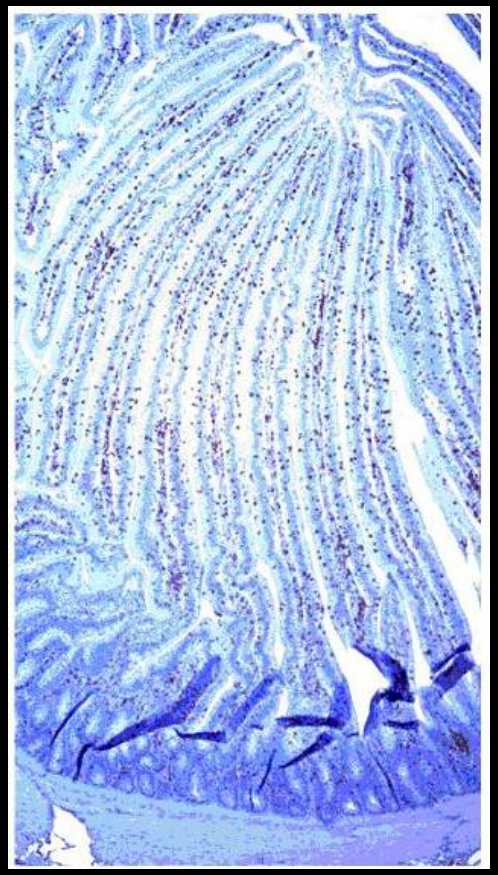
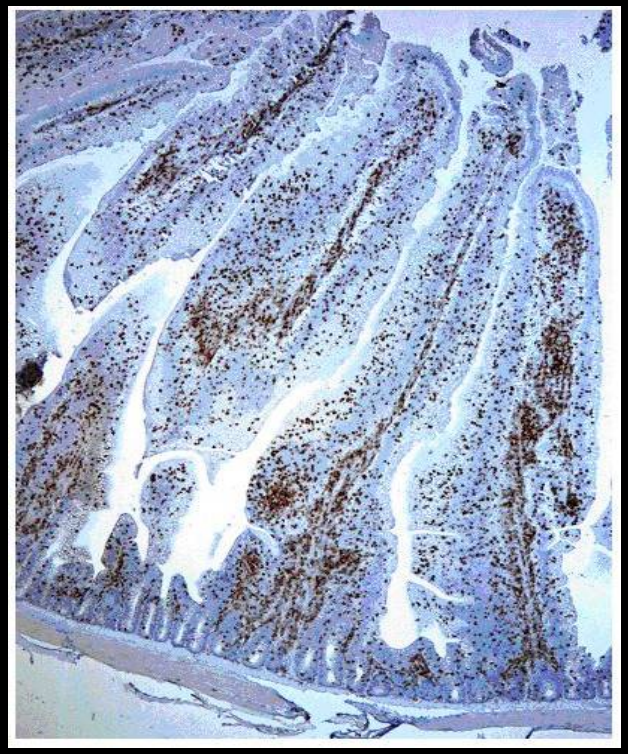
Innate immune response = inflammation

T-lymphocytes immunohistochemistry



healthy

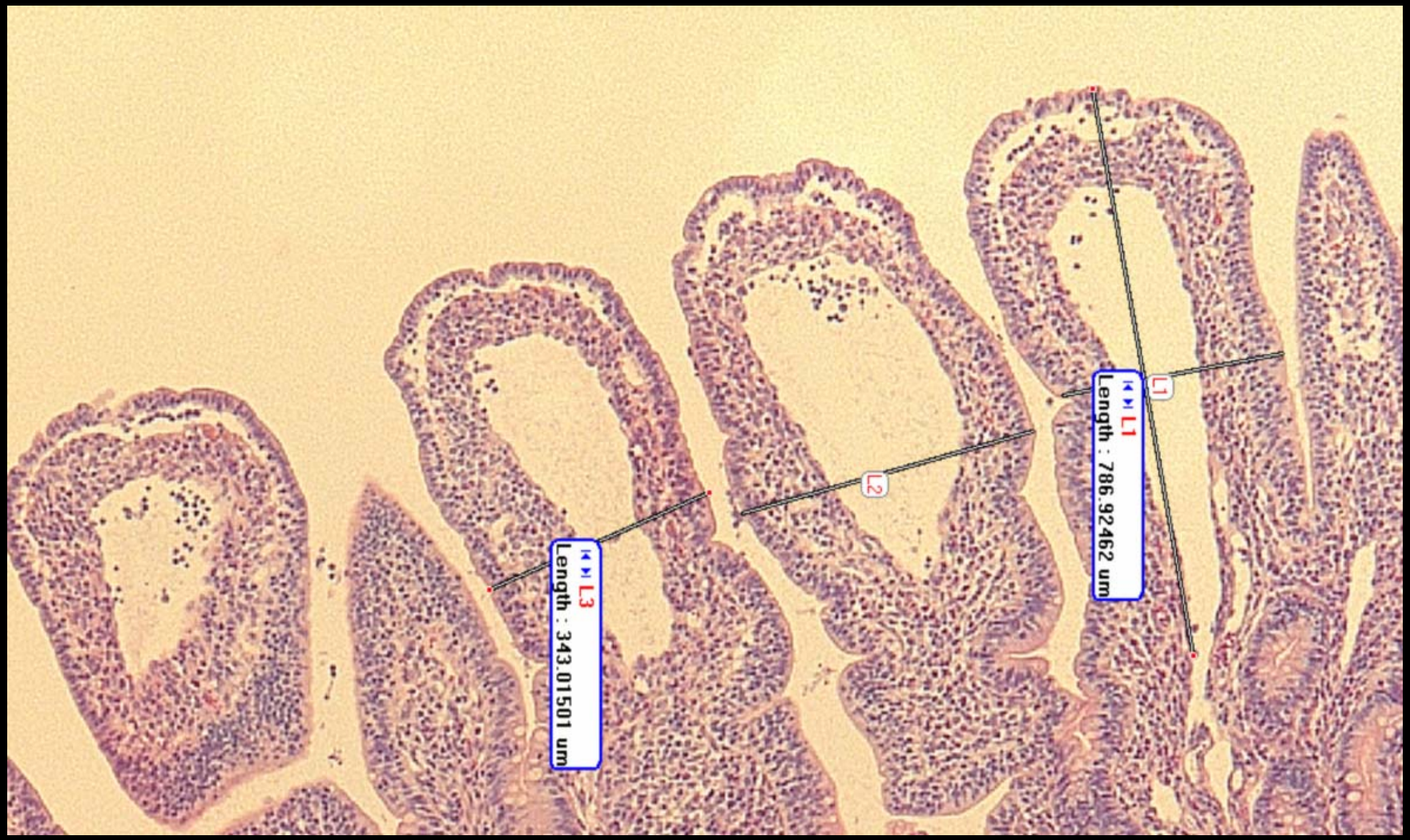


inflammation

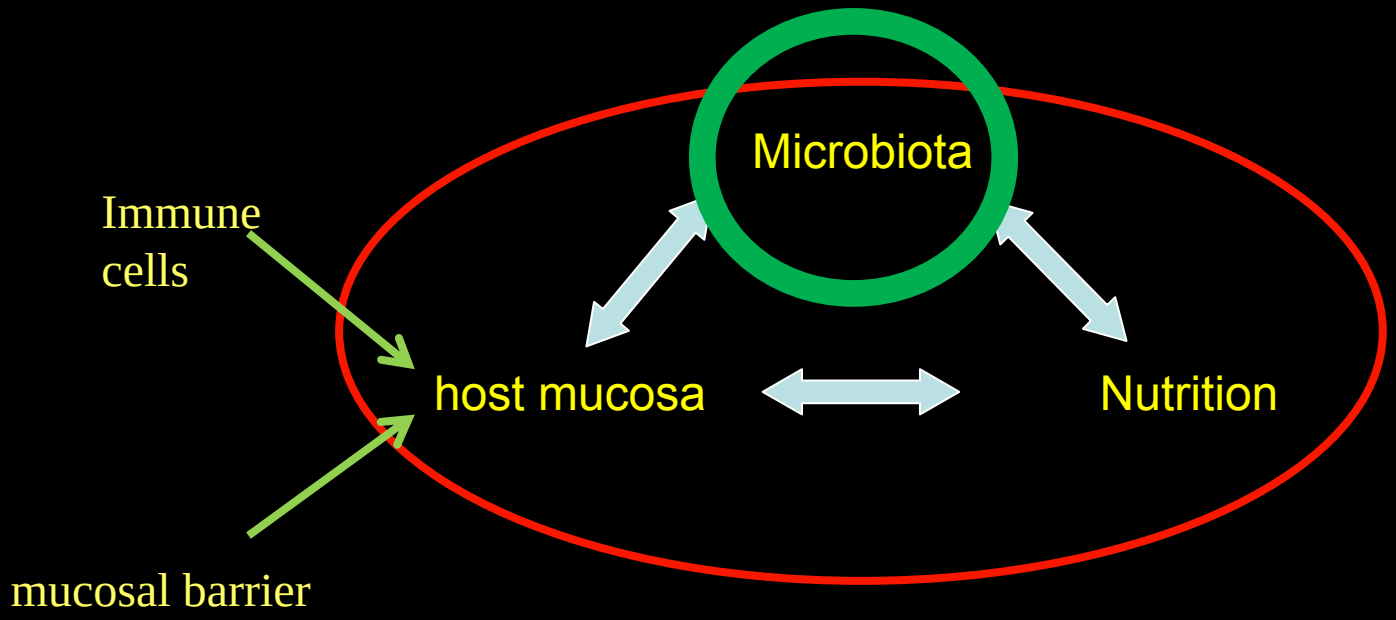
What happens when tolerance mechanisms fail ?

Inflammation = reduced lymph drainage

« leaky gut »



The intestinal ecosystem



It is estimated that the human microbiota contains as many as 10^{14} bacterial cells, a number that is 10 times greater than the number of human cells present in our bodies

Sekirov et al., 2010

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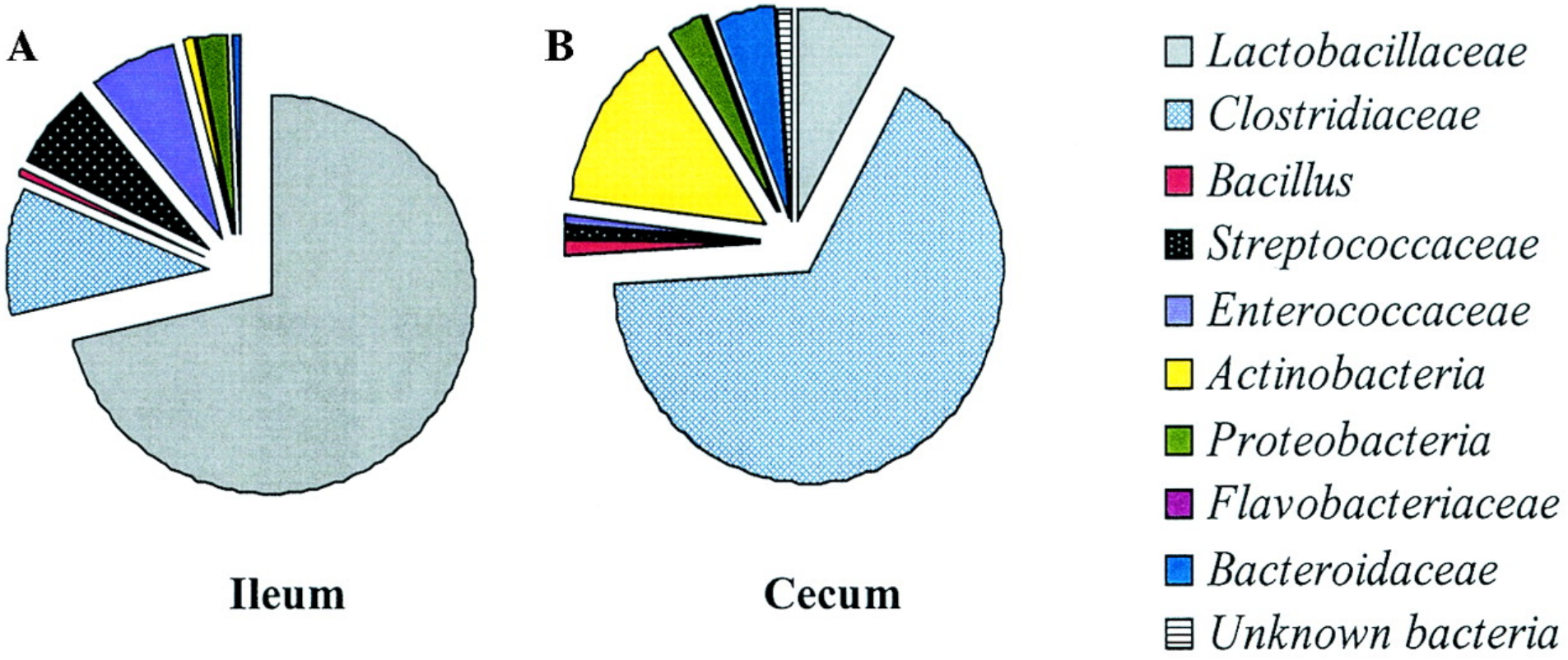
Sekirov et al., 2010

> 35,000 bacterial species

Microbiota composition of the chicken ileum and cecum



(Lu et al., 2003)

Effects of butyrate

- Energy source of colonocytes
- Inhibition of histone deacetylase (Daly & Shirazi-Beechey, 2006)
- Anti-inflammatory (Hamer et al., 2008)
- Multiplication and differentiation of epithelial cells
- Suppression of NF-κB activation (Seguin et al., 2000)
- Increased anti-oxidant capacity in colonocytes (Vanhoutvin et al., 2009)
- Upregulation of PPAR-γ (Kinoshita et al., 2002)
- Increased tight junction proteins
- Induction of neutrophil apoptosis (Aoyama et al., 2010)
- Increased glutathione production by colonocytes (Hamer et al., 2009)

Conclusions

The normal intestinal immune system is characterized by tolerance

Oral tolerance requires vitamin A

Certain feed ingredients like n-3 PUFAs dampen inflammatory response through PPAR-γ activation

A non-hostspecific class of intestinal microorganisms belonging to the phylum of Firmicutes, can protect the colon mucosa against epithelial damage and inflammation.