

The analysis and influence of key regulating factors on our gastrointestinal system and beyond:

**Various scientific based indications
for Bio-available Butyrate**

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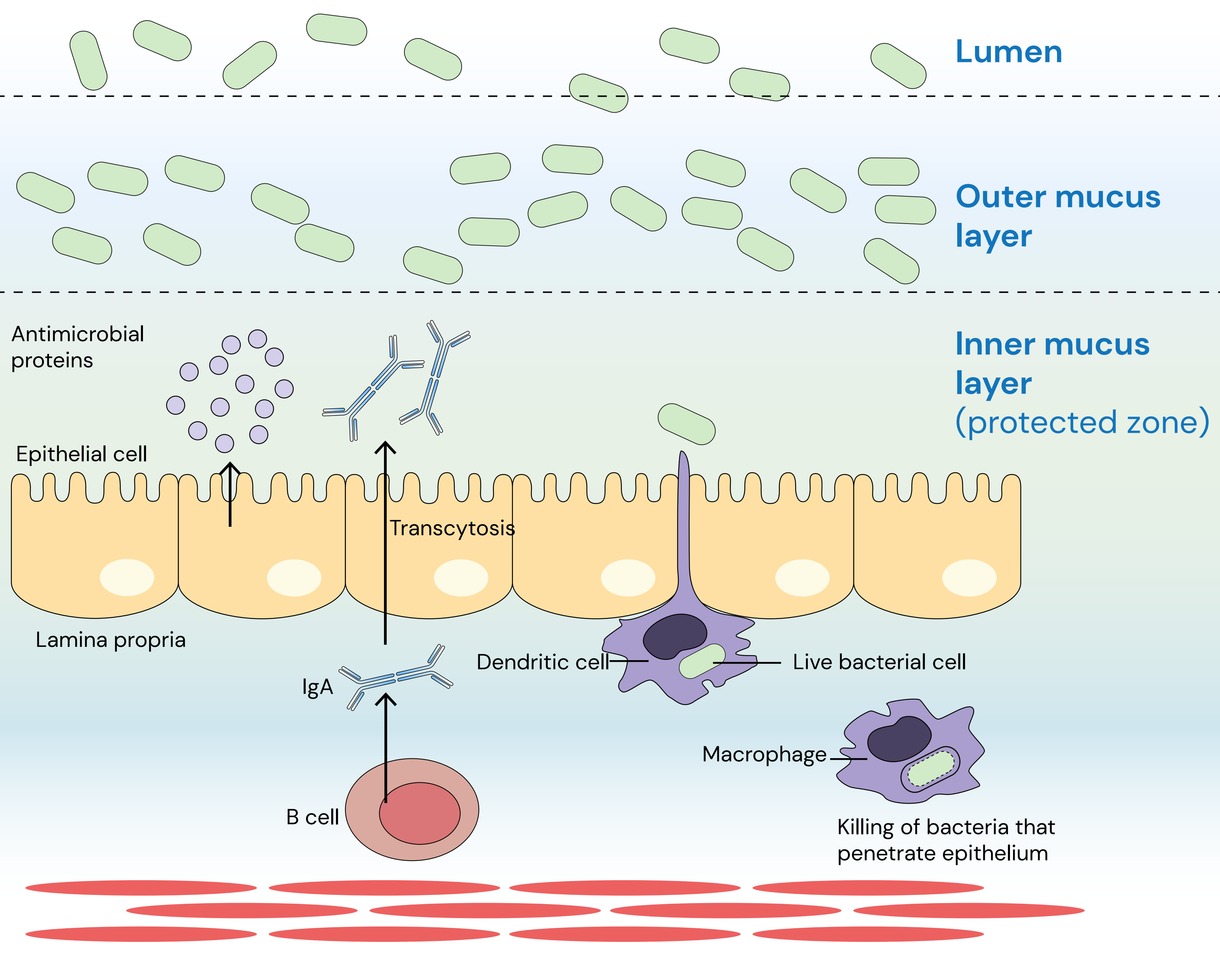

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Goal of this Lecture

- **Introduction on the Gastro-Intestinal Barrier as a multi-layered system**
- **Definition of Butyrate, short chain fatty acid produced by fermentation in the colon**
- **Overview of activities and indications for Butyrate in a technical device that guaranties proper bio-availability:**
 - Gastro-intestinal permeability
 - Immune modulation
 - Communication between gut & brain

What are the primary functions of the gut barrier?

- Water & electrolyte balance
- Prevents influx of pathogens, toxicants and antigens from the lumen of the gut
- Regulates appropriate inflammatory and immune responses



Lumen

Microbial barrier
commensal bacteria

Outer mucus layer

Inner mucus layer
(protected zone)

Chemical barrier
mucus layer

Physical barrier
the epithelium

Immunological barrier
immune cells
of the lamina propria

Muscle layers
smooth muscle intestinal wall

Antimicrobial proteins

Epithelial cell

Transcytosis

Lamina propria

IgA

B cell

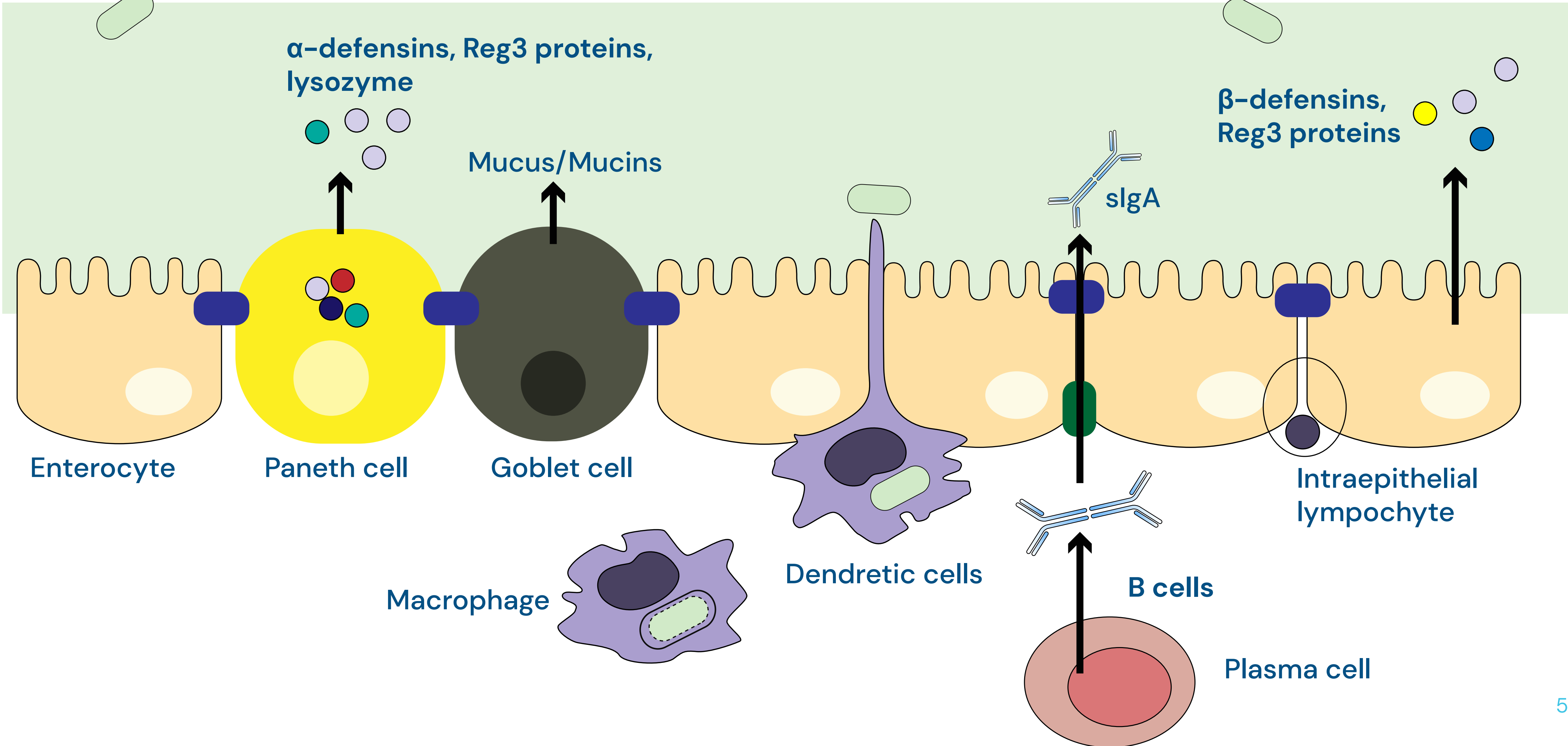
Dendritic cell

Live bacterial cell

Macrophage

Killing of bacteria that
penetrate epithelium

Mucus is a “slimy” material that coats many epithelial surfaces
It is composed chiefly of mucins
Goblet Cells secrete Mucins

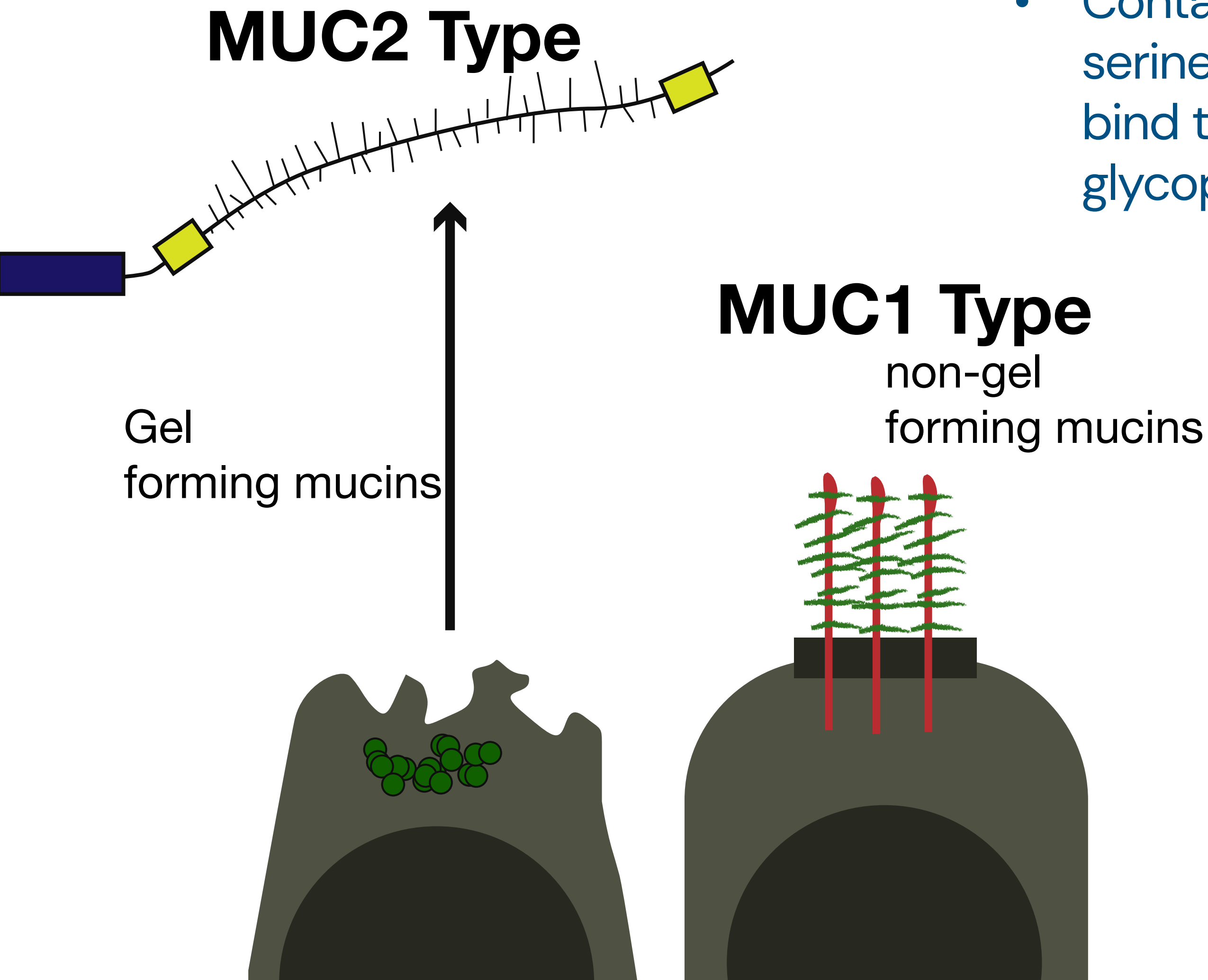


Mucins are highly glycosylated proteins, polymers form a gel-like network

- Mucins are essential to maintain Gut Barrier function, mucins can be compared with biofilm
- Mucins are to the epithelial cells as the biofilm is to bacteria and yeast;
- Prevent direct bacterial binding to epithelial cells
- Mucins are regulated by commensal microbiota within the mucosa

MUC 2 Type

- Secretory mucins
- Being released from the Goblet cells
- Contain amino acids with high concentration serine & threonine, and that is where the glycans bind to form a water attracting network of glycoproteins



MUC 1 type

- The non-secretory mucins
- Forming a gel barrier that protects the delicate epithelial cells

How important are commensal bacteria and their metabolites ?

Example

- Adult patient was given antibiotics for a bad cold
- After the cure she suffered from arthritic-like pain / inflammation in hand/fingers

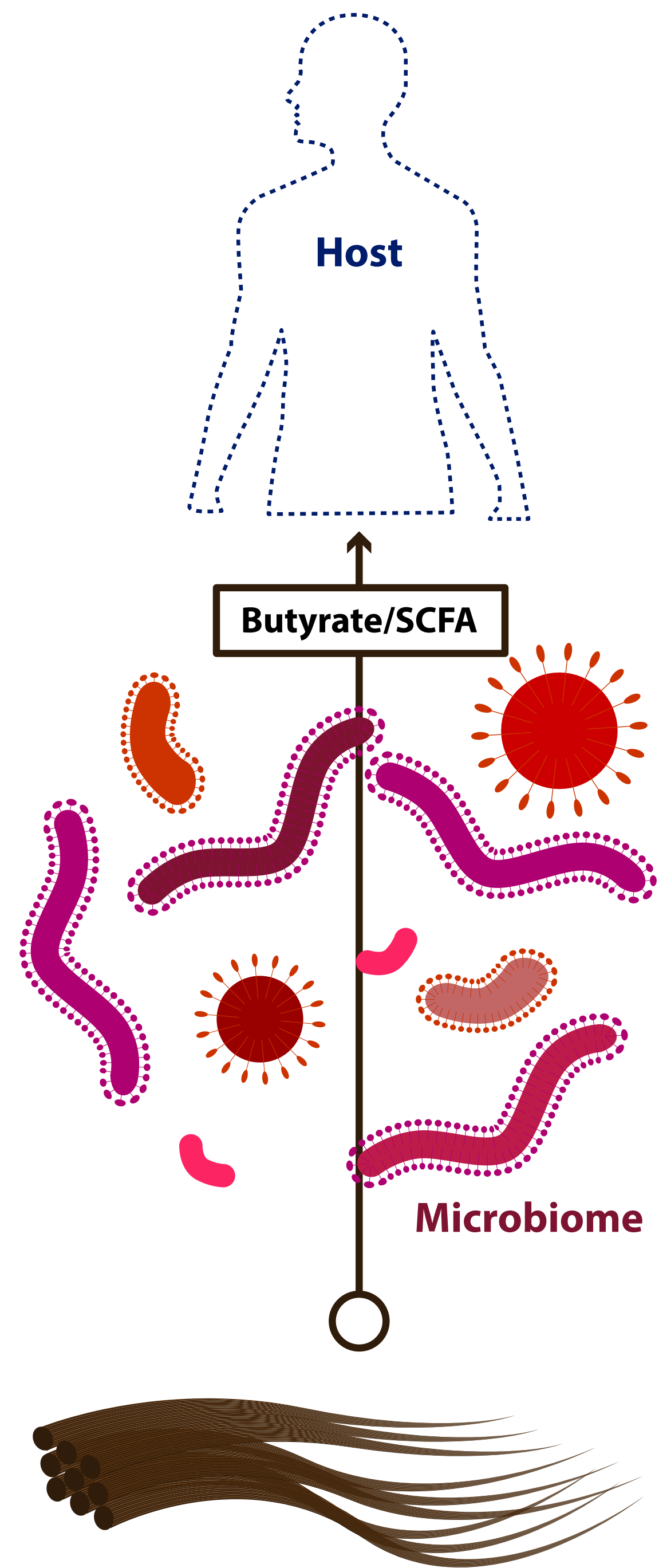
Classic case of insufficiency dysbiosis

- No growth of Bifidobacterium spp.
- No growth of lactobacillus spp.
- Very poor growth of commensal Enterococcus spp.
- Marginal growth of the commensal Clostridium spp.

Loss of key commensals

→ compromised intestinal barrier system

→ Inflammation



Major issue
= Decrease in Butyrate production

butyrate is a major intestinal messenger

Loss of butyrate

= Microbial-host cross talk compromised

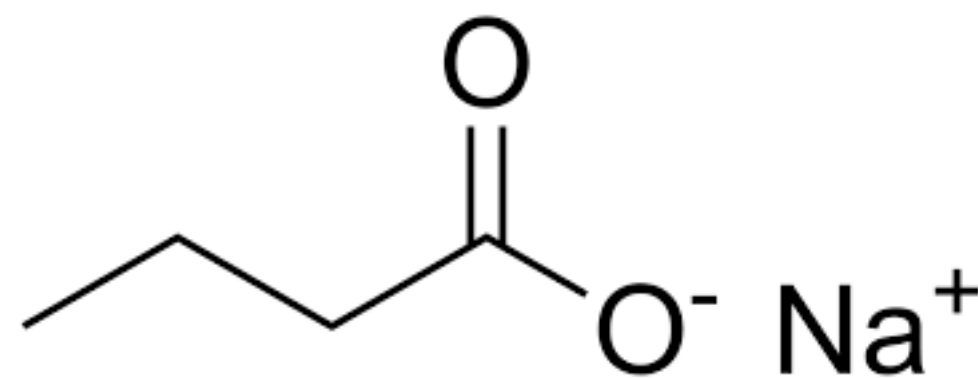
Compromised permeability

+ We get colonization of imbalanced Flora

How is butyrate formed?

1. From host prebiotic

Mucin harvesting bacteria that release glycans
= mucin derived glycans are fermented by other bacteria
to form butyrate



What bacteria produce butyrate?

- **Clostridium spp.** have a key regulatory role
= major butyrate producers – initiating that cross talk
- **Fecalibacterium prausnitzii**

- We have a decreased amount of Clostridium spp. in colorectal cancer and IBD versus controls
- The more fibers, vegetables and beans we eat, the more abundant Clostridium spp. are
- Vs. we also have 5 very pathogenic spp. like C difficile
– **THE MAJORITY OF CLOSTRIDIUM spp. ARE NOT BAD**

2. From exogenous prebiotics (fibers)

onions, garlic, asparagus, leeks, yams, chicory root, bananas

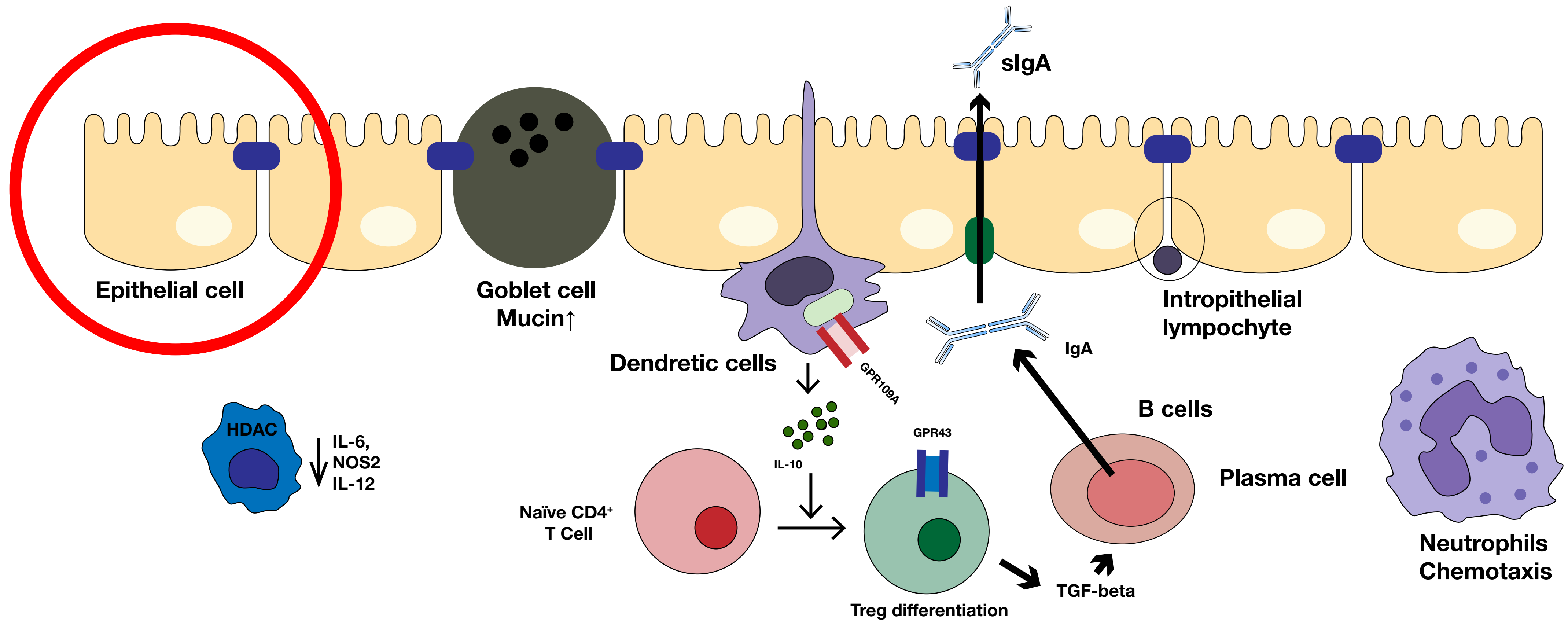


What is the role of butyrate and SCFA's?

Butyrate, acetate, propionate

Fuel to renew the intestinal epithelial cells (IEC)

IEC need to be renewed every 3-5 days



Microbial–host cross talk: “ the host listens to butyrate”

=Butyrate impacts epigenetics

= Butyrate modifies genetic material

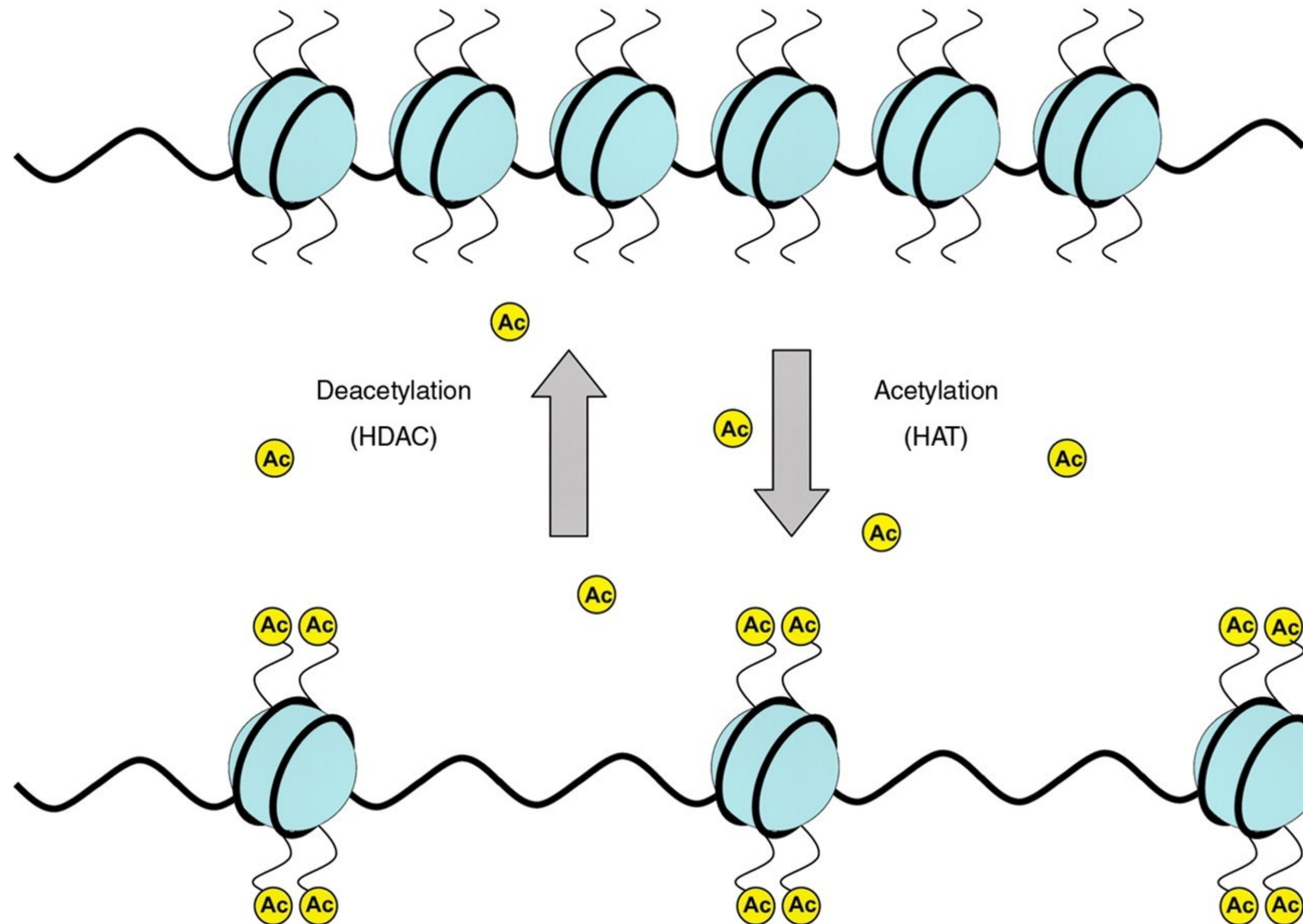
→ impact on gene expression and transcription

Epigenetics most often involves changes that affect gene activity and expression

Such effects may result from external or environmental factors, or be part of normal development.

Examples of mechanisms that produce such changes are DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying DNA sequence.

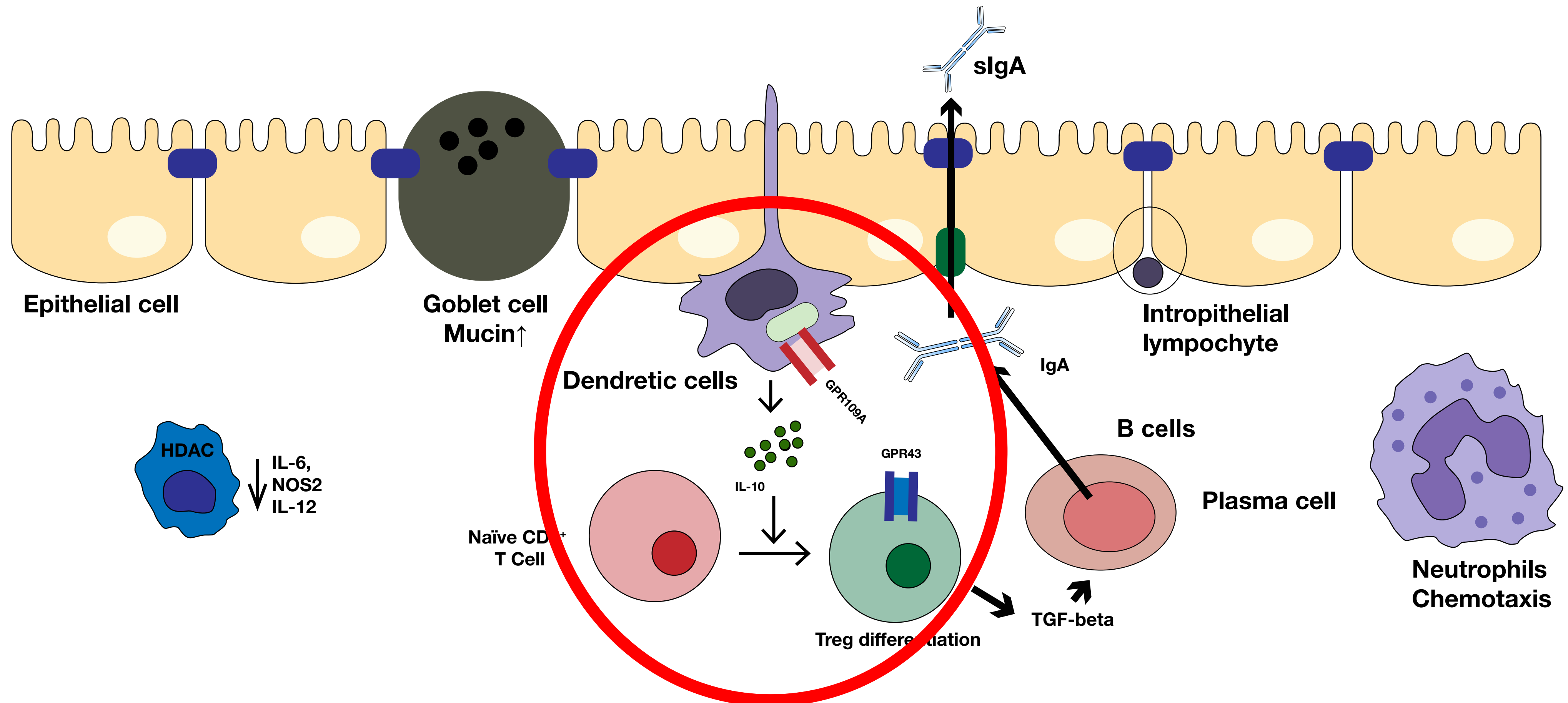
Immune modulation / anti inflammation on local level:
Butyrate inhibits HDAC (histone deacetylase)
– this modification is changing the gene expression



Gene expression is modified in Dendritic Cells

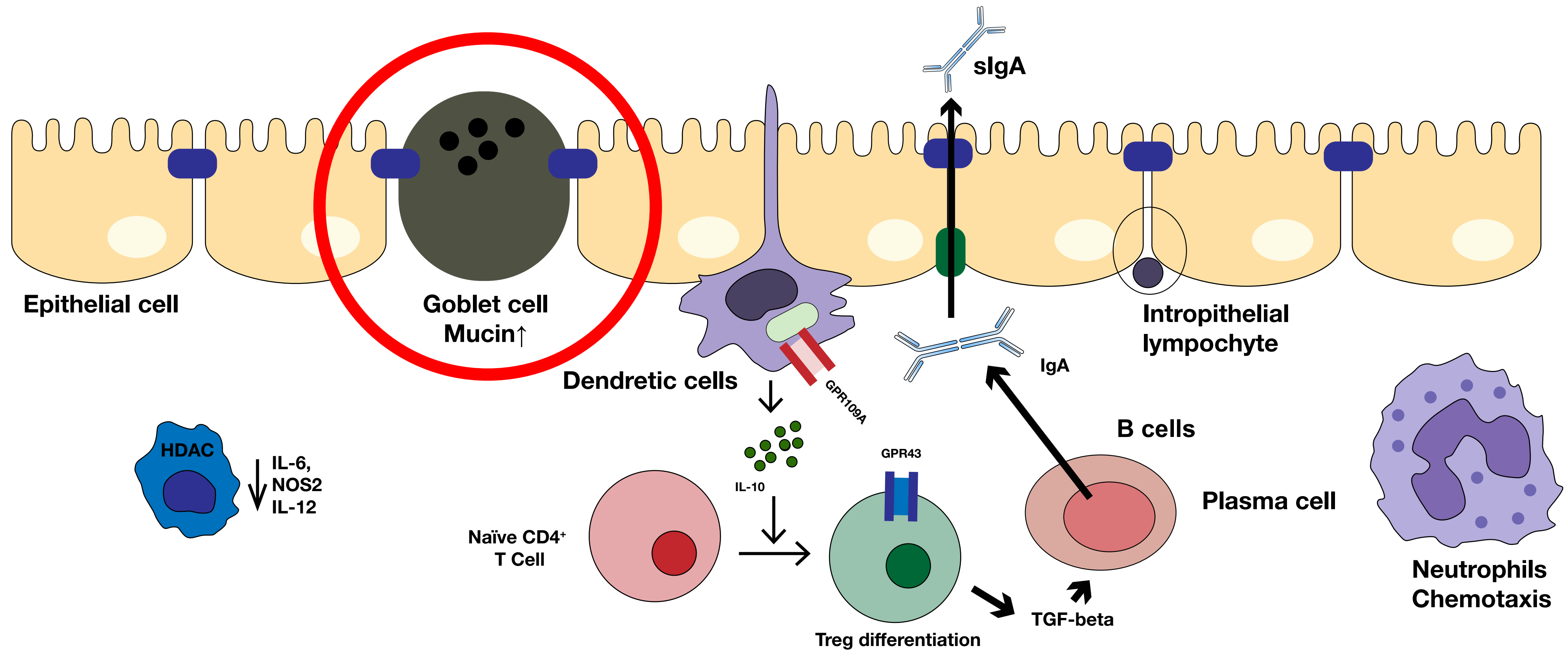
IL-6 is suppressed = more IL-10

More differentiation to T regs



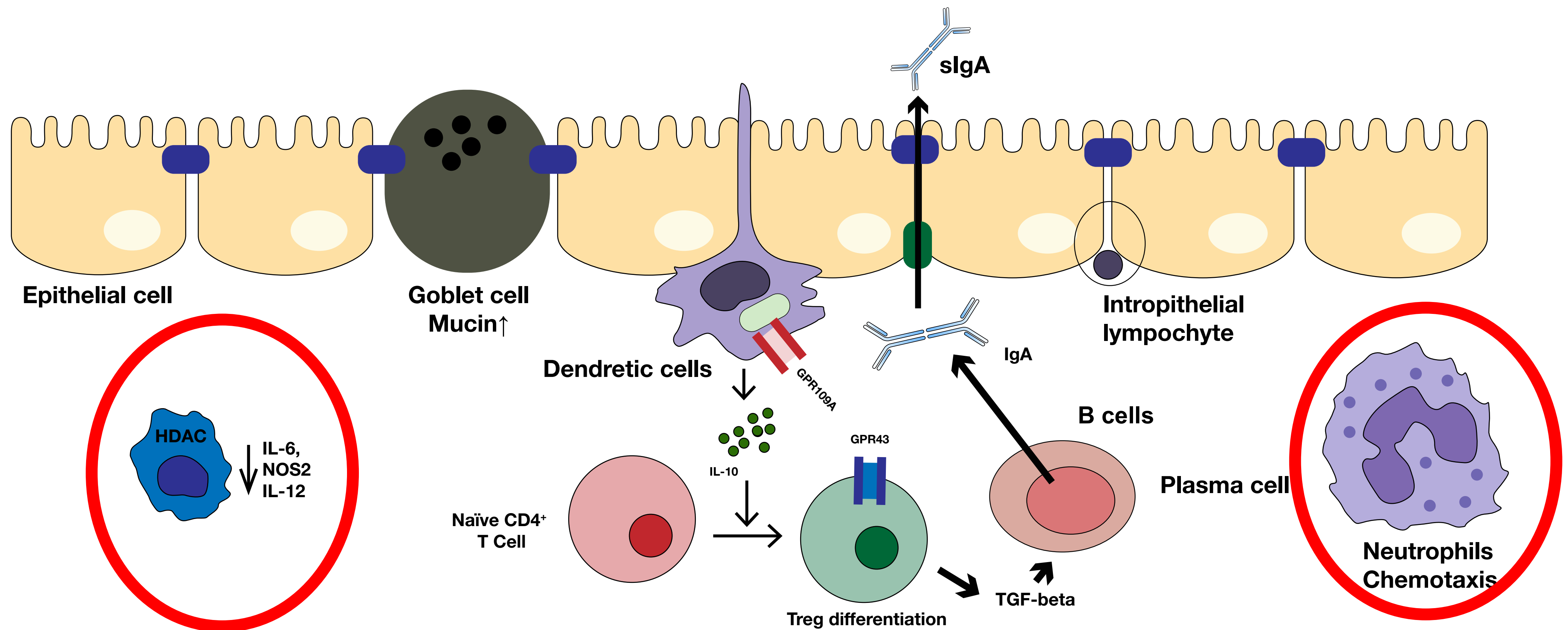
Differentiation of Goblet Cells and mucus formation

More mucin is a better immune defense against invading pathogens



Butyrate modulates the immune response in macrophages what makes macrophages more tolerant towards commensal bacteria

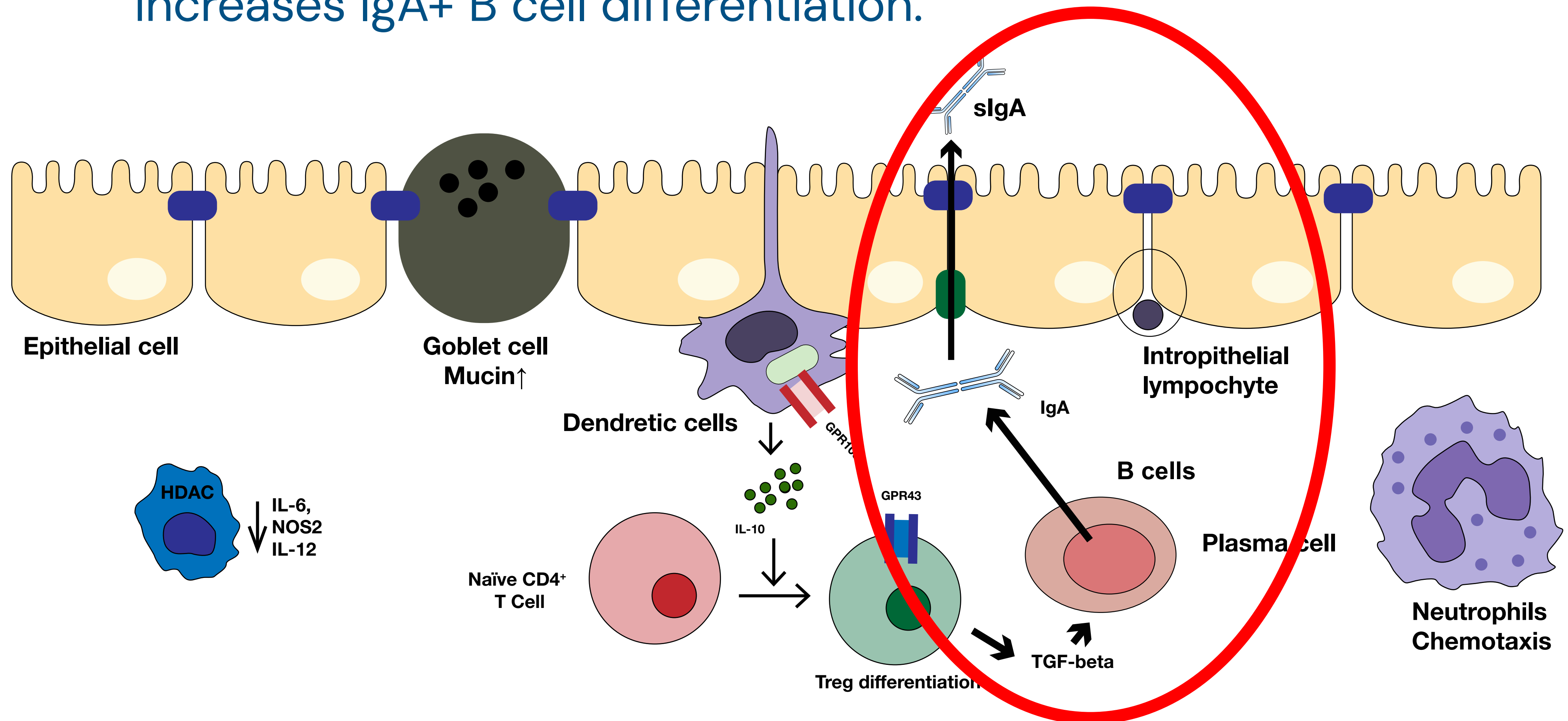
Butyrate affects neutrophil chemotaxis anti inflammation on local level



sIgA

TGF-beta produced by Treg cells drives naïve B cells to differentiate into IgA-producing cells.

IL-21 from Th17 cells accentuates the effect of TGFb and increases IgA+ B cell differentiation.



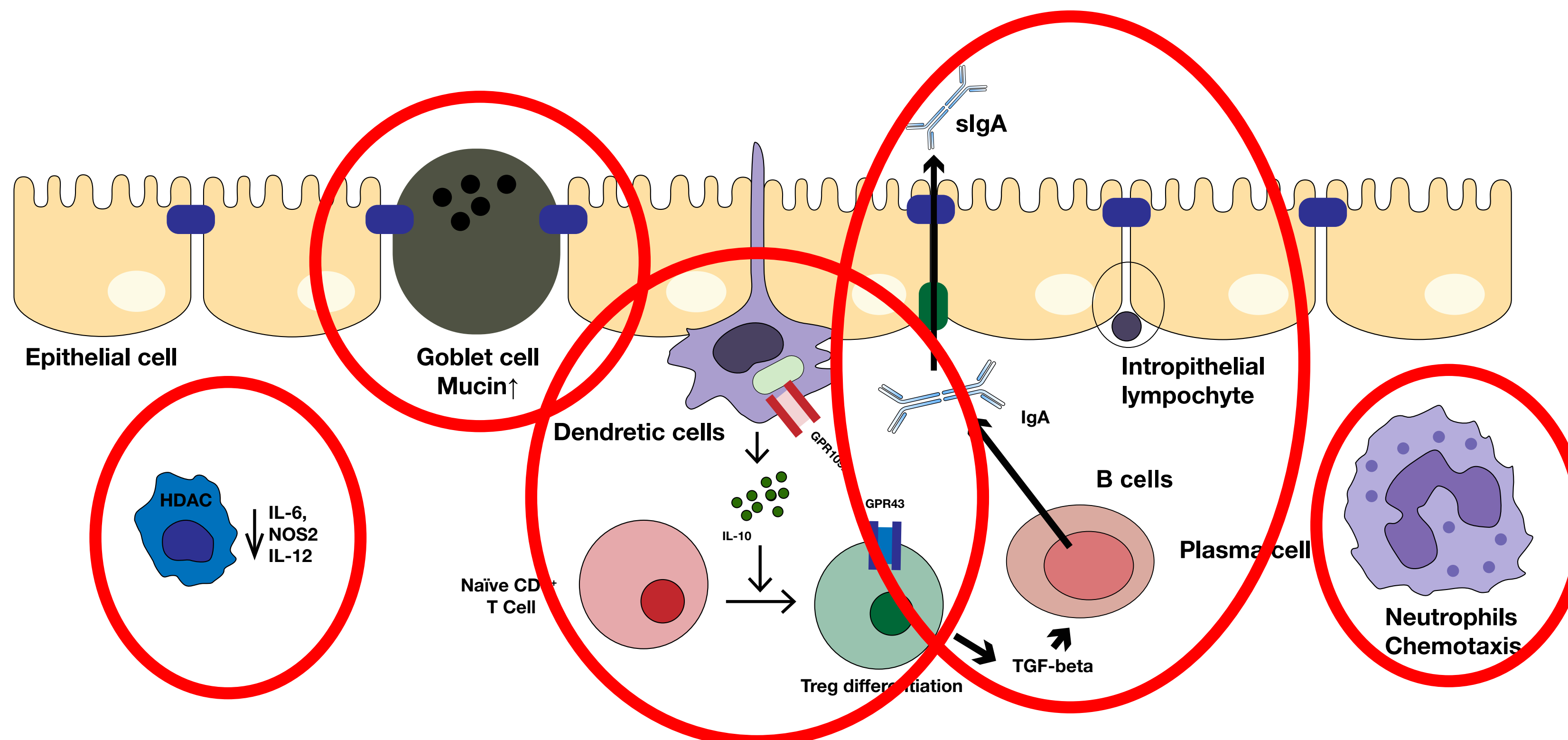
Fuel to renew epithelial cells

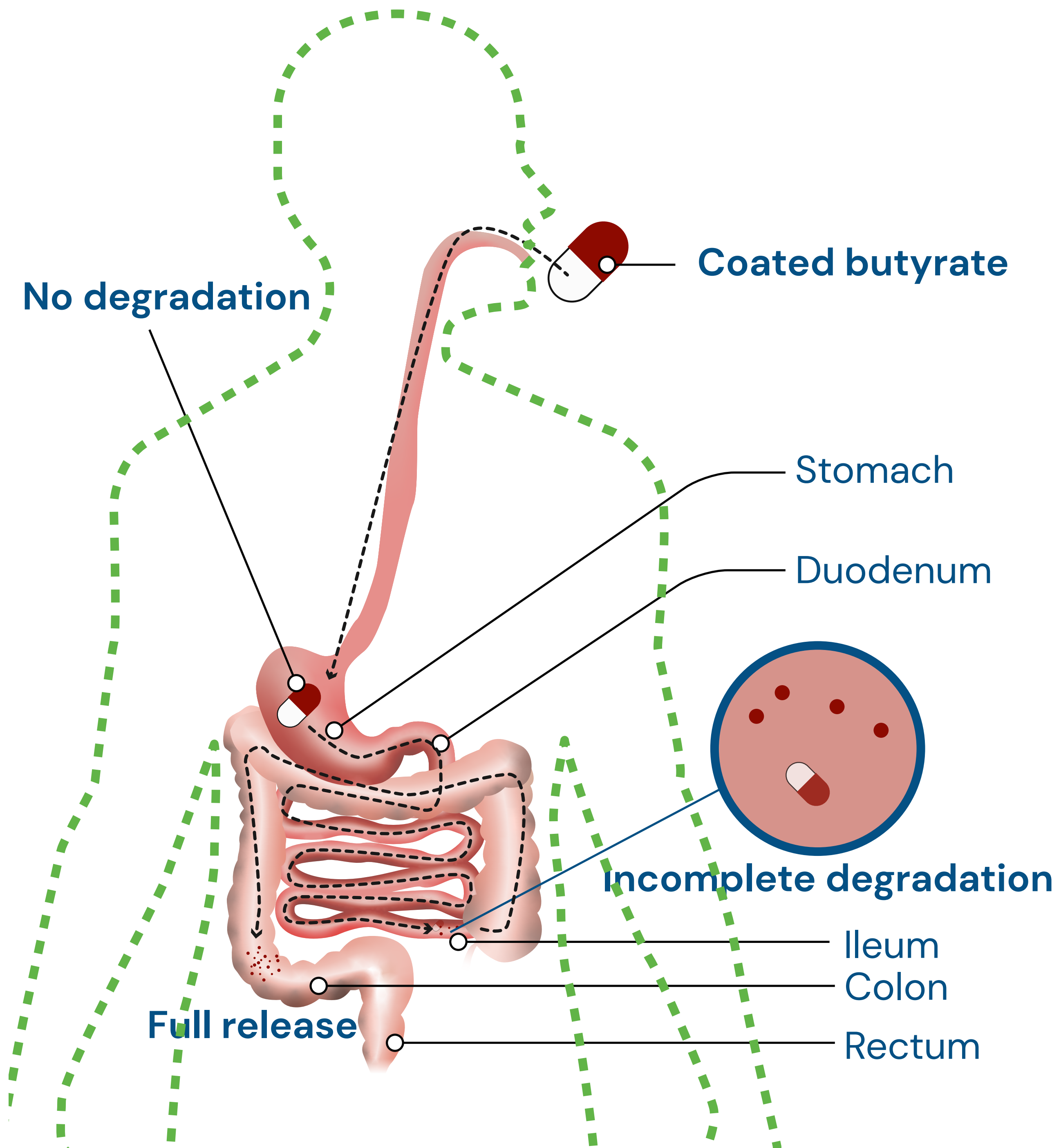
Impact on dendritic cells,
more IL-10 & T regs

Goblet Cells release more mucins

Macrophages more tolerant towards commensal bacteria

Neutrophil chemotaxis





Supplemental Butyrate needs coating for overall activity on different levels

- To obtain both local and systemic effect
- To avoid a premature release and absorption of butyrate
- To ensure complete release of the active ingredient at a time comparable to the oro-ilear transit time

Donohoe, Dallas R., et al. "Microbial regulation of glucose metabolism and cell-cycle progression in mammalian colonocytes." *PloS one* 7.9 (2012).

Donohoe, Dallas R., et al. "The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon." *Cell metabolism* 13.5 (2011): 517-526.

Sanderson, Ian R. "Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium." *The Journal of nutrition* 134.9 (2004): 2450S-2454S.

Arpaia, Nicholas, et al. "Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation." *Nature* 504.7480 (2013): 451-455.

Chang, Pamela V., et al. "The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition." *Proceedings of the National Academy of Sciences* 111.6 (2014): 2247-2252.

Vinolo, Marco AR, et al. "Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils." *The Journal of nutritional biochemistry* 22.9 (2011): 849-855.

Usami, Makoto, et al. "Butyrate and trichostatin A attenuate nuclear factor κ B activation and tumor necrosis factor α secretion and increase prostaglandin E2 secretion in human peripheral blood mononuclear cells." *Nutrition research* 28.5 (2008): 321-328.

Kim, Ha-Jung, et al. "Clinical efficacy and mechanism of probiotics in allergic diseases." *Korean journal of pediatrics* 56.9 (2013): 369.

Marchix, Justine, Gillian Goddard, and Michael A. Helmuth. "Host-gut microbiota crosstalk in intestinal adaptation." *Cellular and molecular gastroenterology and hepatology* 6.2 (2018): 149-162.

Cao, Anthony T., et al. "Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis." *The Journal of Immunology* 189.9 (2012): 4666-4673.

Keubler, Lydia M., et al. "A multihit model: colitis lessons from the interleukin-10-deficient mouse." *Inflammatory bowel diseases* 21.8 (2015): 1967-1975.

Wilson, Mark S., et al. "Colitis and intestinal inflammation in IL10^{-/-} mice results from IL-13R α 2-mediated attenuation of IL-13 activity." *Gastroenterology* 140.1 (2011): 254-264.

Matt, Stephanie M., et al. "Butyrate and dietary soluble fiber improve neuroinflammation associated with aging in mice." *Frontiers in immunology* 9 (2018): 1832.

Bourassa, Megan W., et al. "Butyrate, neuroepigenetics and the gut microbiome: can a high fiber diet improve brain health?" *Neuroscience letters* 625 (2016): 56-63.

Huuskonen, Jari, et al. "Regulation of microglial inflammatory response by sodium butyrate and short chain fatty acids." *British journal of pharmacology* 141.5 (2004): 874-880.

Roda, Aldo, et al. "A new oral formulation for the release of sodium butyrate in the ileo-cecal region and colon." *World Journal of Gastroenterology: WJG* 13.7 (2007): 1079.

1. GI repair

In various situations

- **Modern life-style factors are causing increased intestinal barrier permeability**
As a result we see more translocation of bacteria and toxins which is causing low-grade inflammation

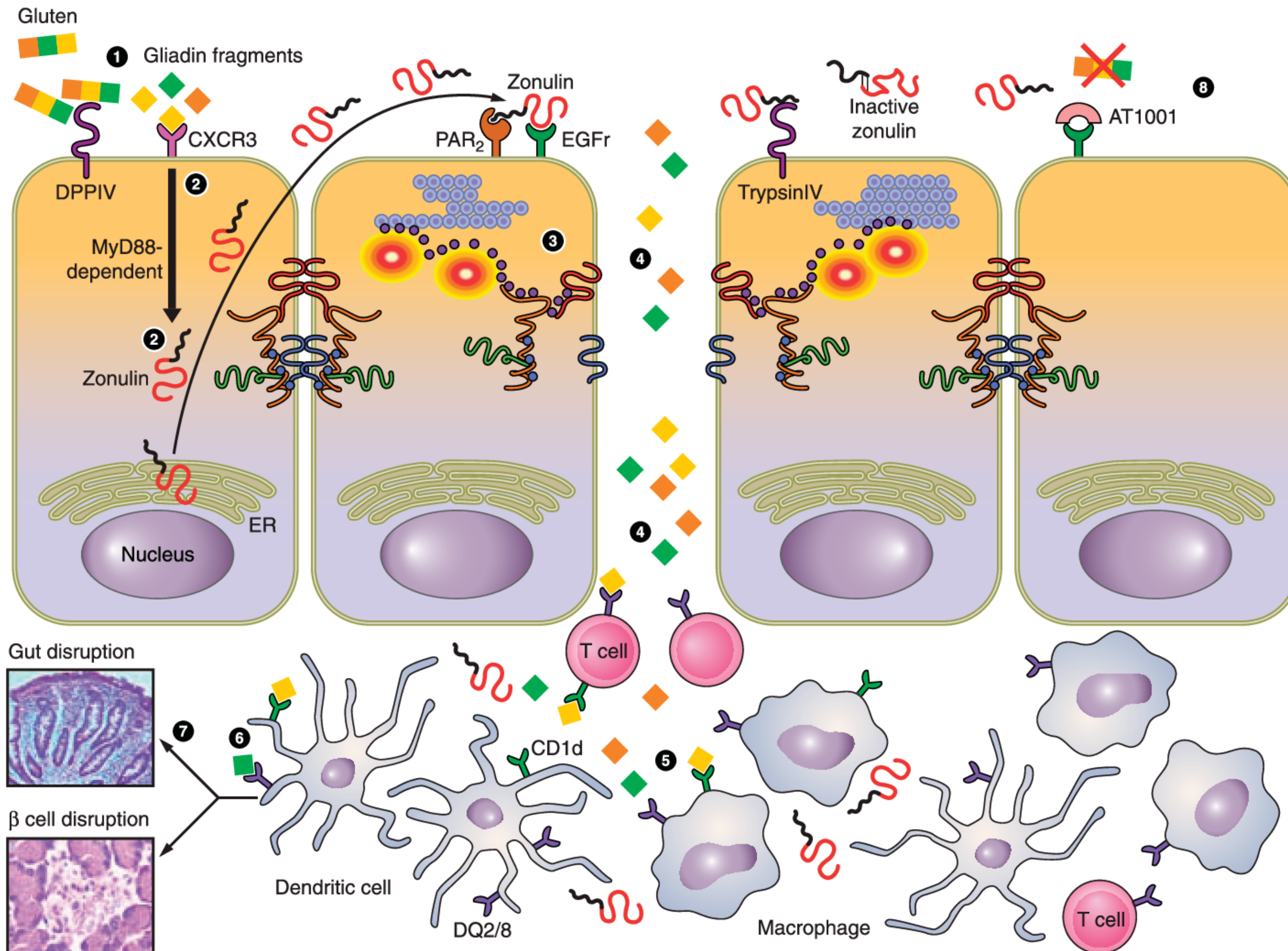
This low-grade inflammation is one of the leading causes of work absence, disability and mortality.

- **Environmental pollution (heavy metals , mycotoxins ...)**
Deoxynavanol (DON) and Cadmium, individually and in combination, increased paracellular permeability in a dose dependent manner. Exposure was associated with a decrease in occludin

de Punder, Karin, and Leo Pruimboom. "Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability." *Frontiers in immunology* 6 (2015): 223.

Luo, Su, et al. "In vitro and in vivo effects of a mycotoxin, deoxynivalenol, and a trace metal, cadmium, alone or in a mixture on the intestinal barrier." *Environment international* 132 (2019): 105082.

Gliadin-induced Zonulin Release → Damaged tight junctions



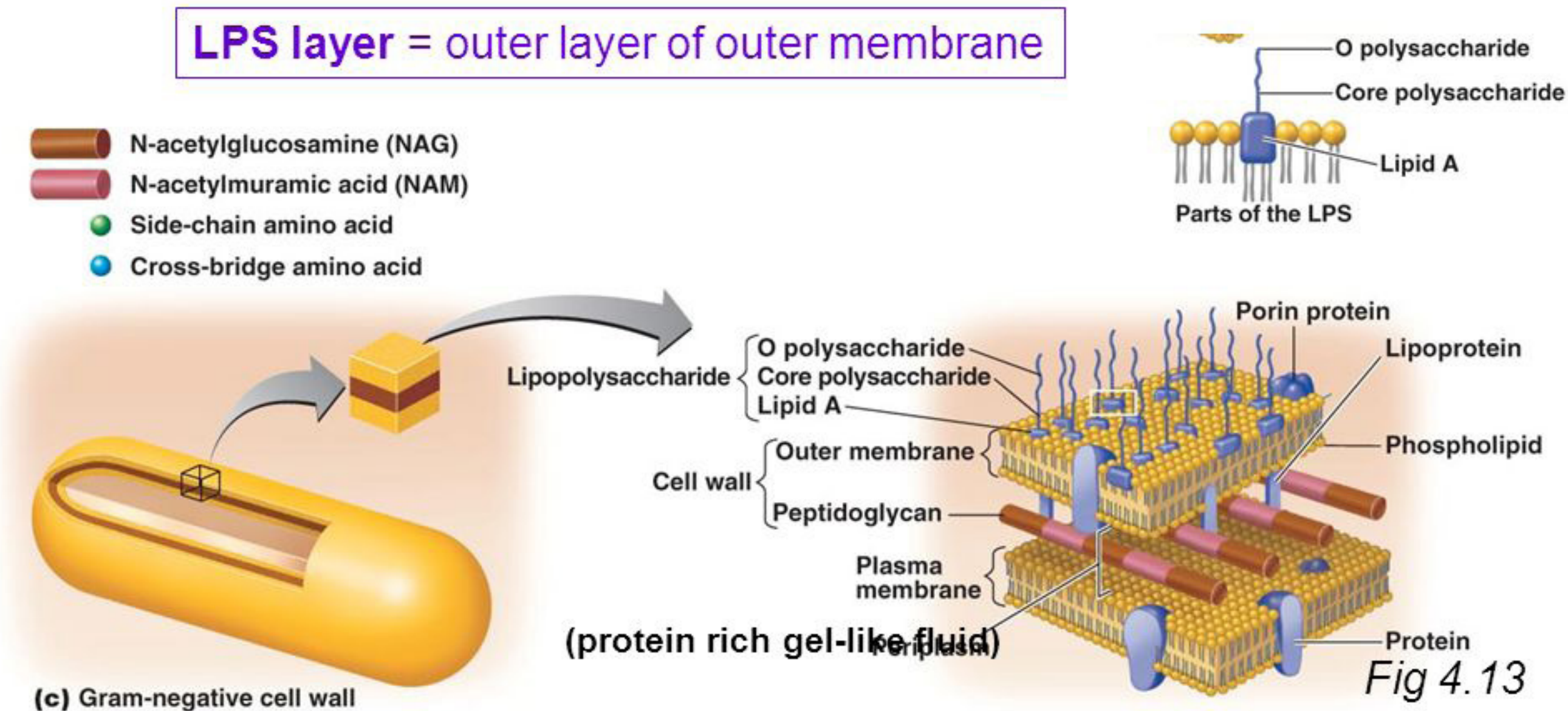
LPS

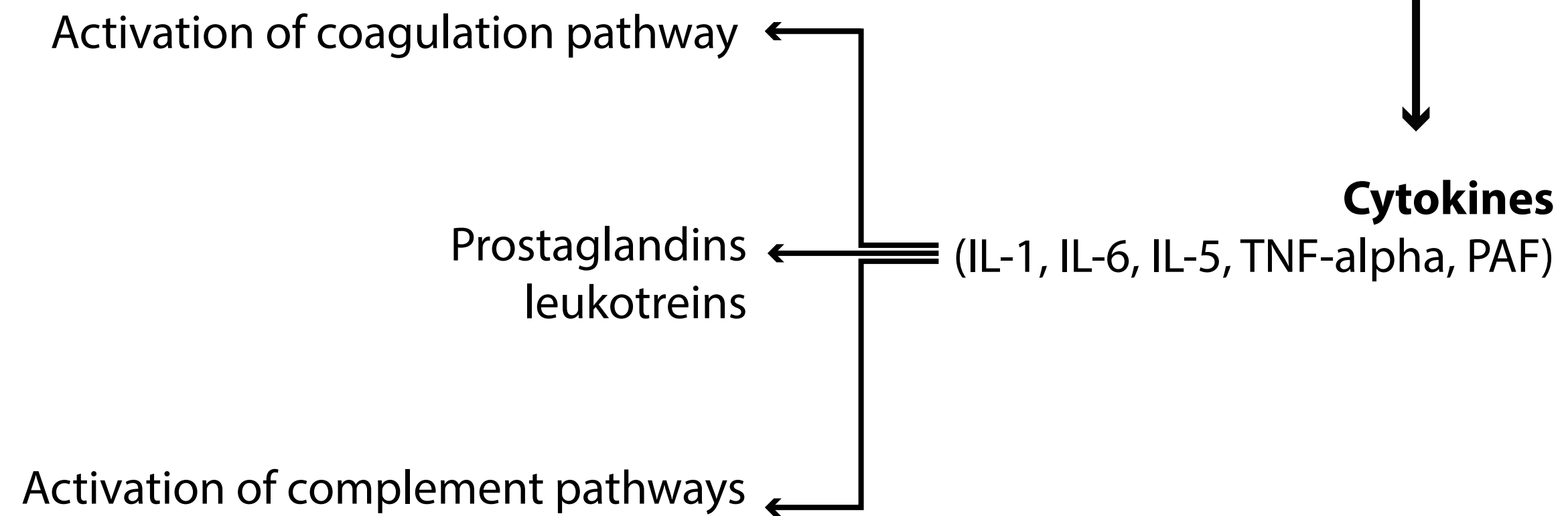
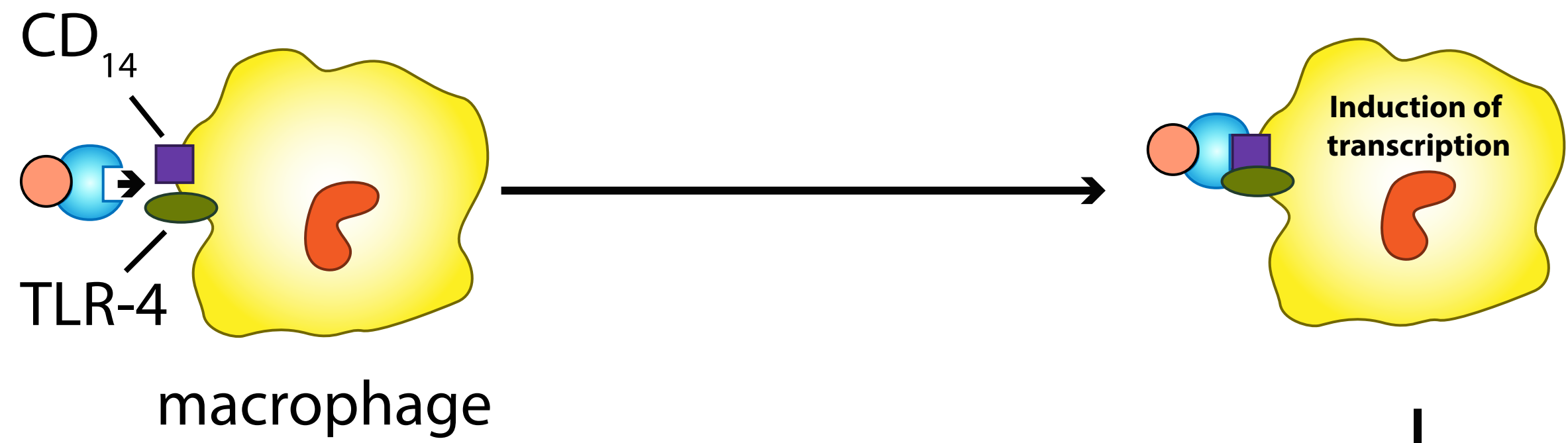
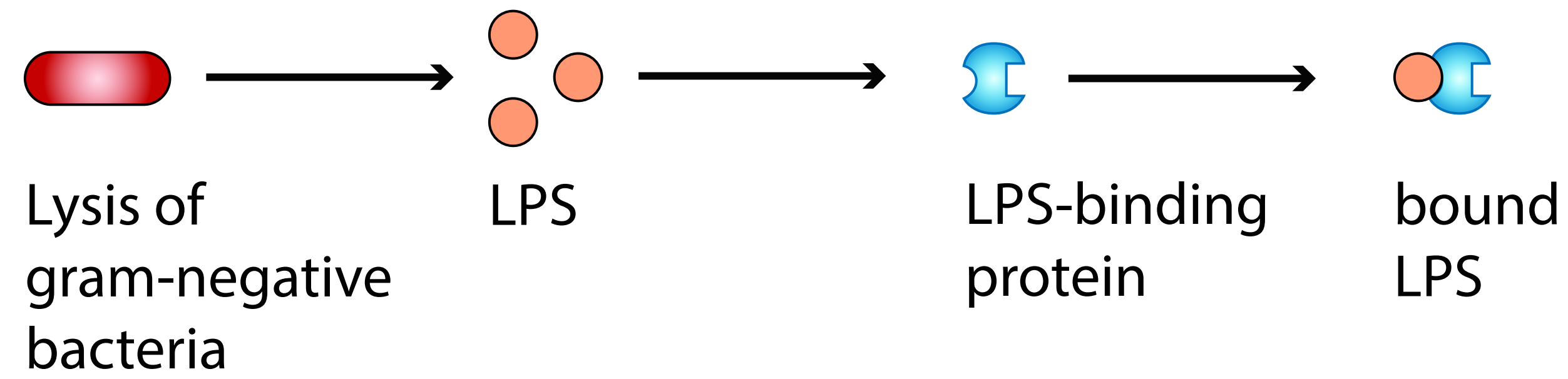
= The major part of the outer cell membrane of Gram-negative gut bacteria

Gram-negative Cell Wall

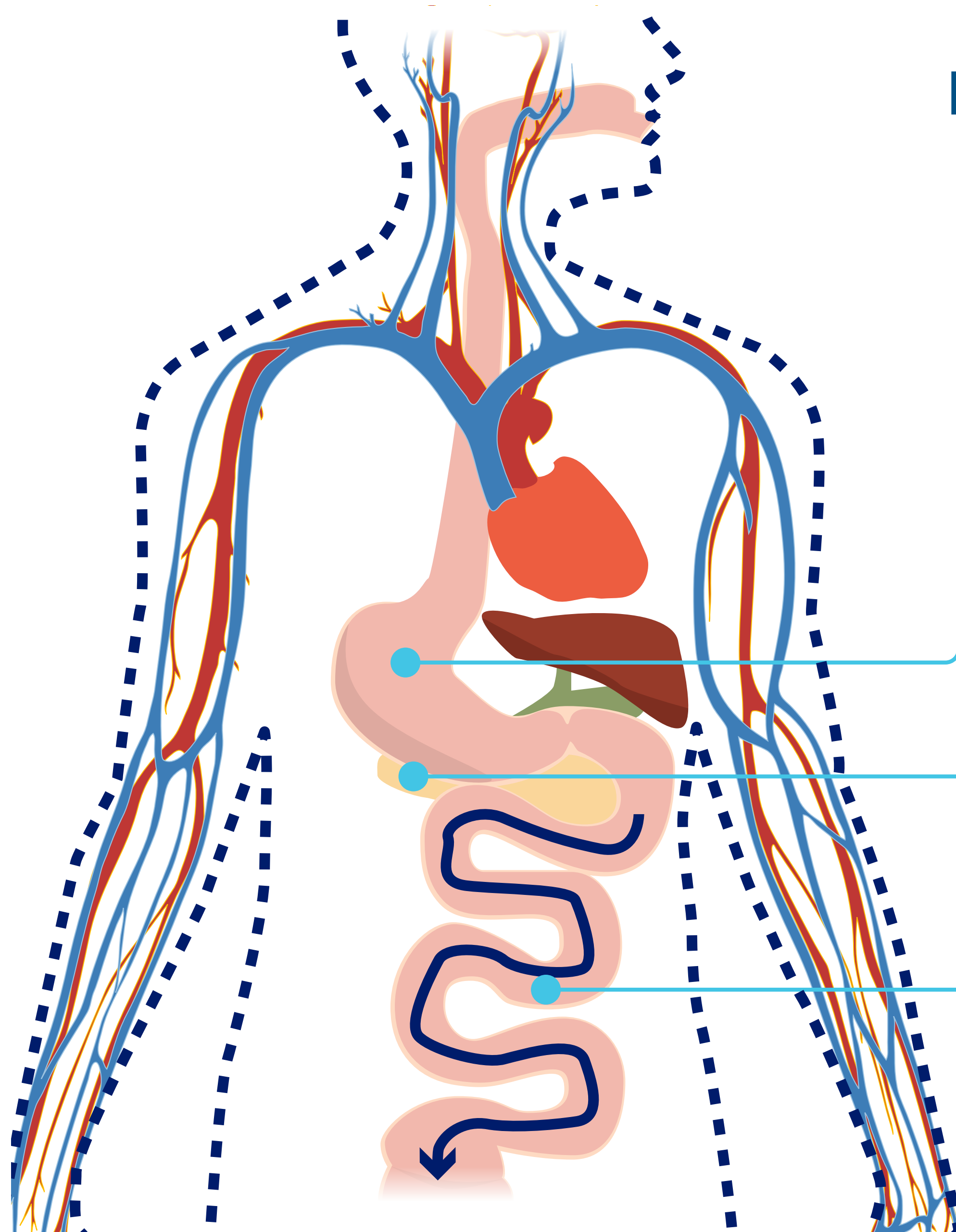
Lipid A of LPS acts as **endotoxin**; **O polysaccharides** are antigens for typing, e.g., *E. coli* **O157:H7**

Gram neg. bacteria are less sensitive to medications because outer membrane acts as additional barrier.





Manage intestinal barrier



Optimize gastric acid level

- Prevents pathogenic overgrowth
- First line defense
- Essential for activation of the pancreas to secrete digestive enzyme
- polypeptides → amino acids (↓auto-immune reactivity)

Enzyme complex to optimize digestion

(including gluten modifying enzymes)

Targeted released Glutamine & cofactors

Heal the mucosal lining and tight junction optimizing (pH 6-7)

- ↓inflammation
- Improve the synthesis of sIgA by the intestinal lymphocytes

Butyrate coated

- Immune tolerance intestinal & systemic
- sIgA barrier
- Mucus barrier

Guttae Pepsini



indication

Stomach acid deficiency
 Poor digestion
 Intestinal malabsorption
 Rebuilds intestinal pH

dosage

3 x 10 – 20 drops per day at the start of each meal,
 dilute in water and swallow immediately.

packaging

30 ml per bottle

composition

(amount per 30 drops)

Purified water	5,3 ml
Glycerol	10 ml
Hydrochloric acid HCl 37%	2,7 ml
Pepsine	2 ml

Please find our referenced version on the professional section of our website.
 All information is exclusively aimed at and released to an audience of health care professionals.

Gluten DPP IV Complex



indication DPP-IV proteolytic enzyme complex.
Breaks down proline residues in Gluten and decreases the intestinal immune reaction
Intolerance for gluten and/or casein.
Indigestion, gas, bloating, constipation and diarrhea.

dosage 3 x 1 caps per day at the beginning of each meal.

packaging 90 vegecaps per container

composition (amount per 3 vegecaps)	Protease IV	60 mg
	Lactase	60 mg
	Protease (zuur en neutraal)	70,35 mg
	Amylase	30 mg
	Maltodextrine	24,45 mg
	Gluco-amylase	15 mg
	Invertase	6 mg
	Lipase	4,2 mg

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DPP4?

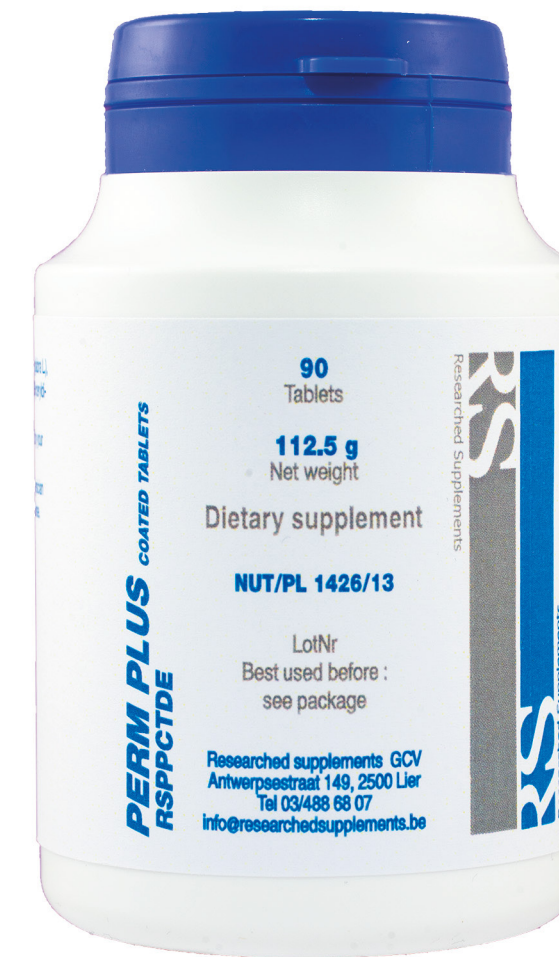
König, Julia, et al. **“Randomized clinical trial: Effective gluten degradation by *Aspergillus niger*-derived enzyme in a complex meal setting.”** Scientific reports 7.1 (2017): 13100.

Gluten is a protein with a high content of proline residues (15%)

Normal enzymes in our GI tract can't break down proline rich sides

This study shows the immunogenicity of Gluten was reduced using DPPIV enzymes

Perm Plus Coated



indication Rebuilding intestinal permeability and immunity with targeted released molecules.

dosage The first month: 3 x 2 tablets per day.
Then take 3 x 1 tablet per day 20 min. before food.

packaging 90 tablets per container

composition (amount per 3 tablets)	L-Glutamine	975 mg
	N-Acetyl-D - Glucosamine	375 mg
	N-Acetylcystein	300 mg
	Liquorice root powder (Glycyrrhiza Glabra L.)	255 mg
	Gamma oryzanol	180 mg
	L-Carnosine	60 mg
	Zinc (as zinc bisglycinate and zinc methionin)	22,5 mg

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Butyflam Coated

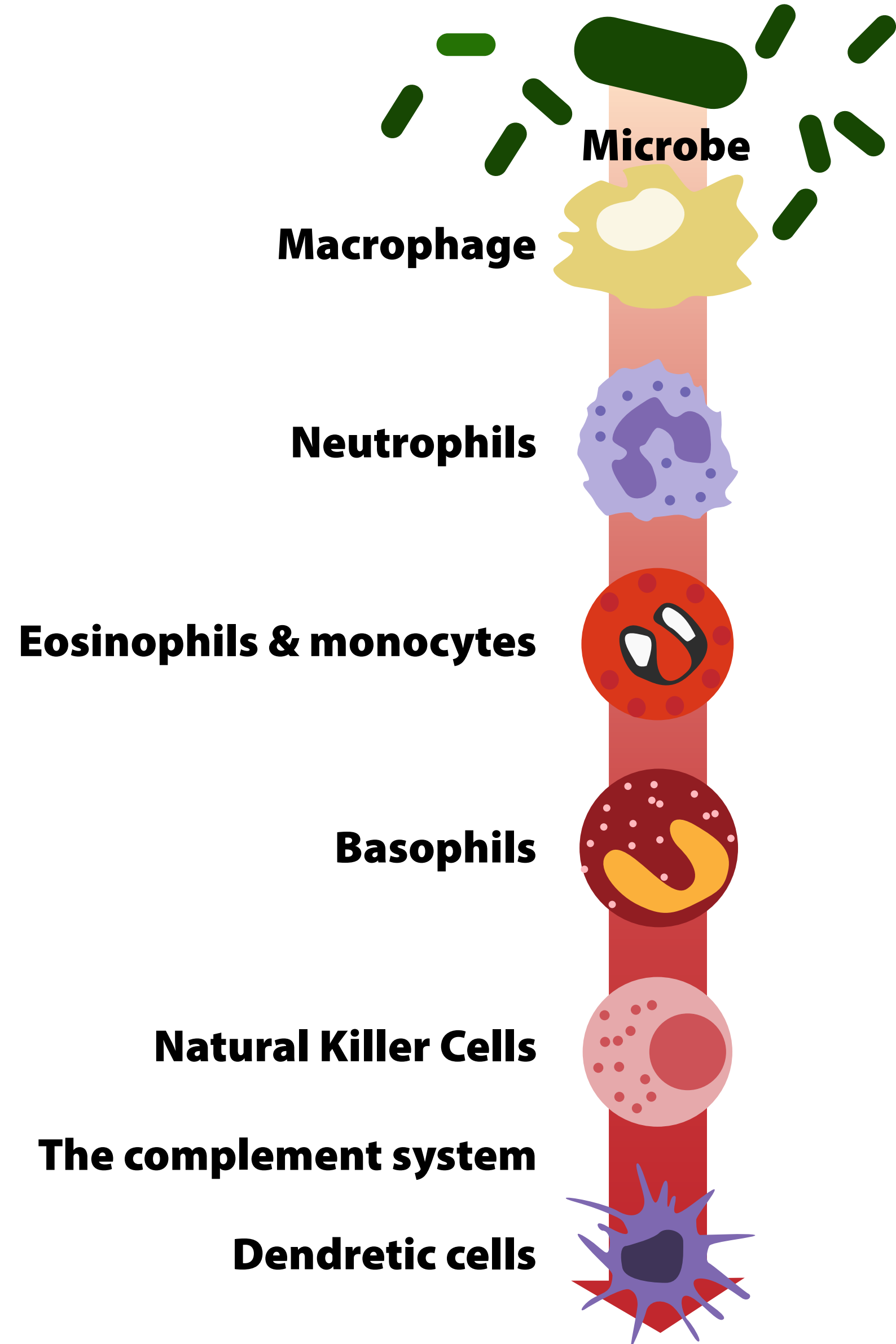
Butyrate is a short-chain fatty acid produced by the intestinal bacteria through fermentation of non-digestible fibers. Butyflam Coated delivers bio-available levels of butyrate in our intestines to guarantee immune tolerance and avoid excessive inflammation or auto-immune reactions.

indication	Neuroinflammation Immune modulating (T reg + IL-10 anti-inflammation) Remodeling intestinal barrier function
dosage	3 x 2 caps per day, 20 minutes before meals
packaging	180 coated caps per container
composition (amount per 6 caps)	Butyrate - 3000 mg

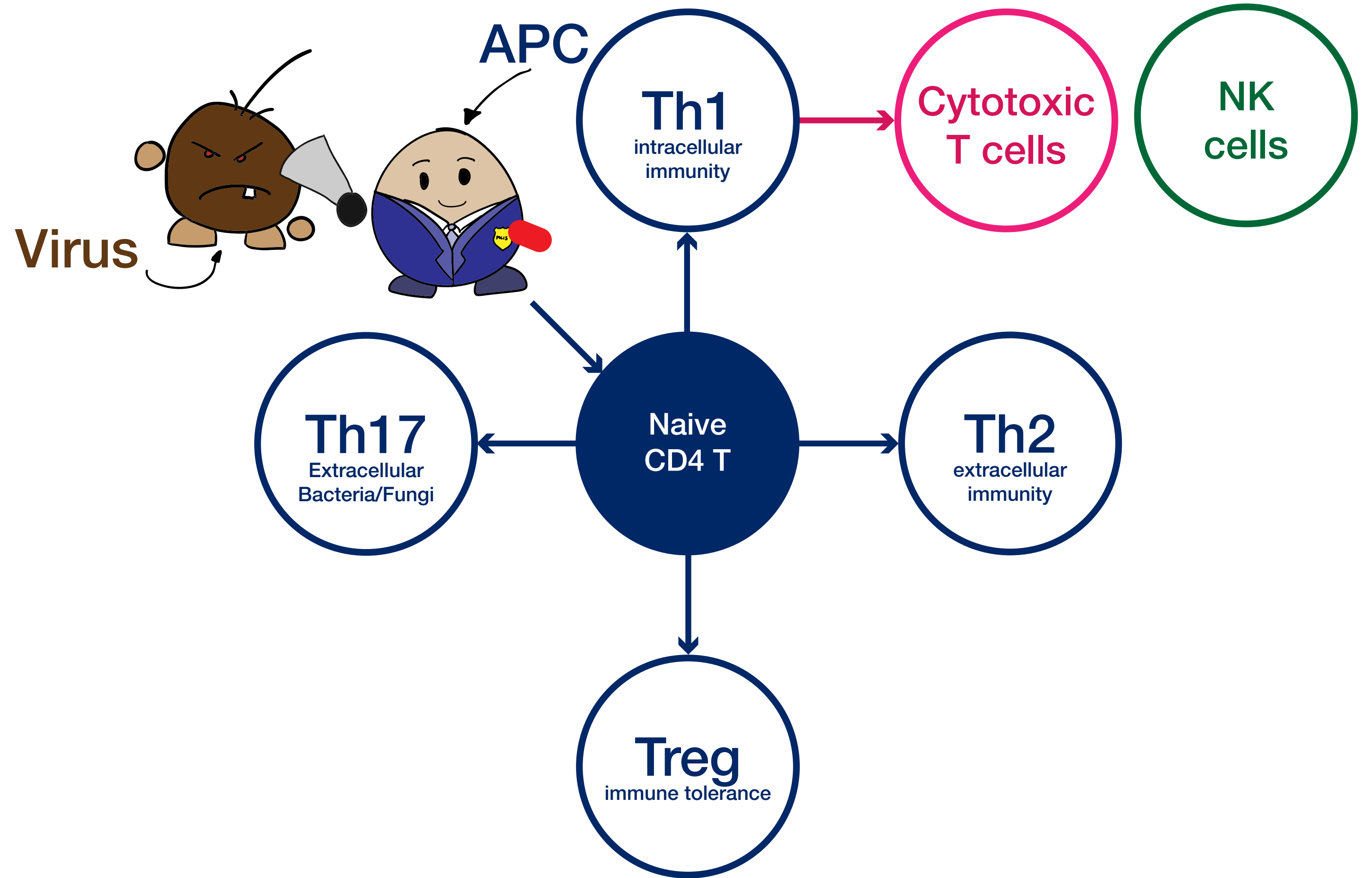
2. Immune modulation in Auto-Immunity or excessive immune response

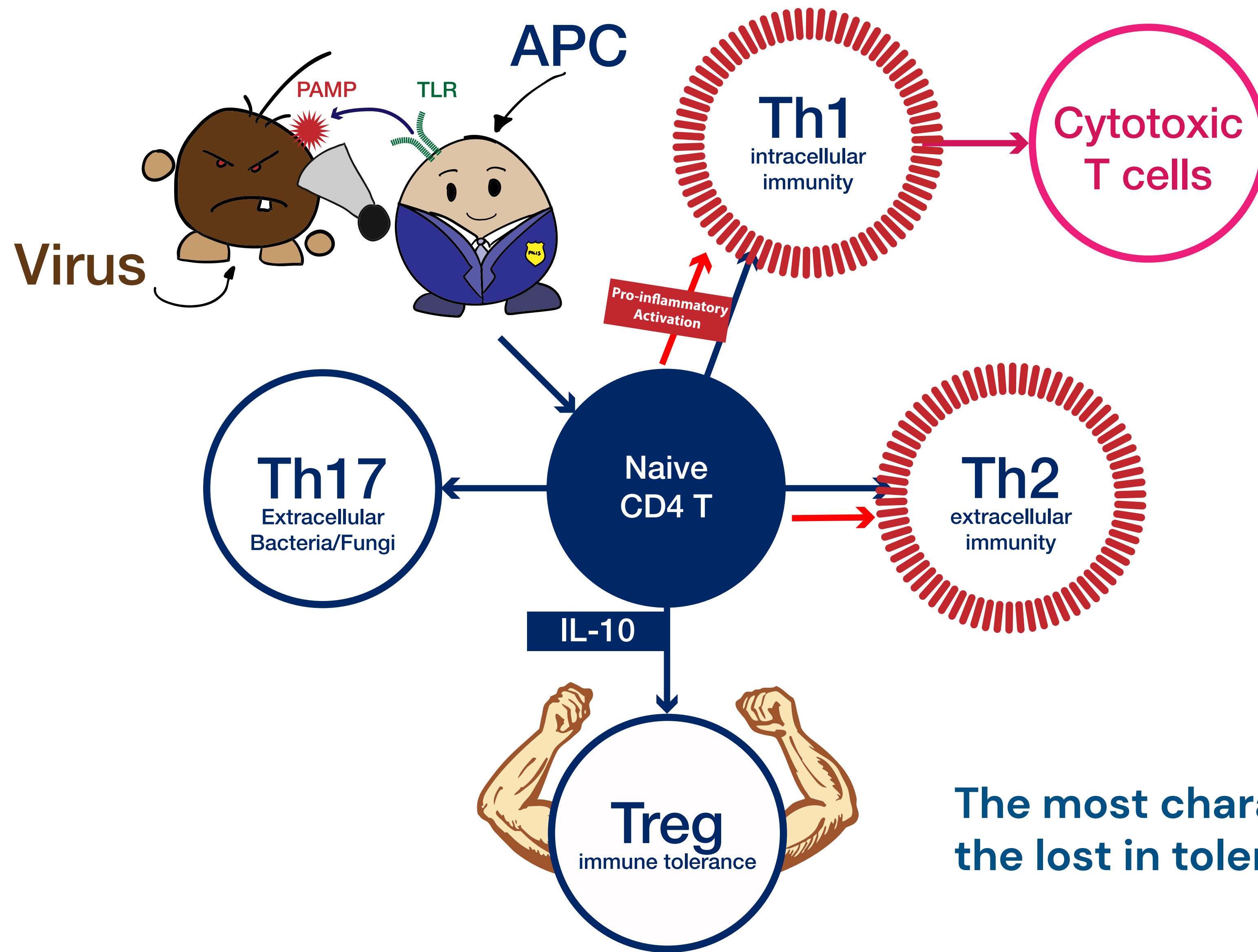
Microbe → **Innate Immune System** → **Adaptive Immune System**

Innate Immunity



Adaptive Immunity



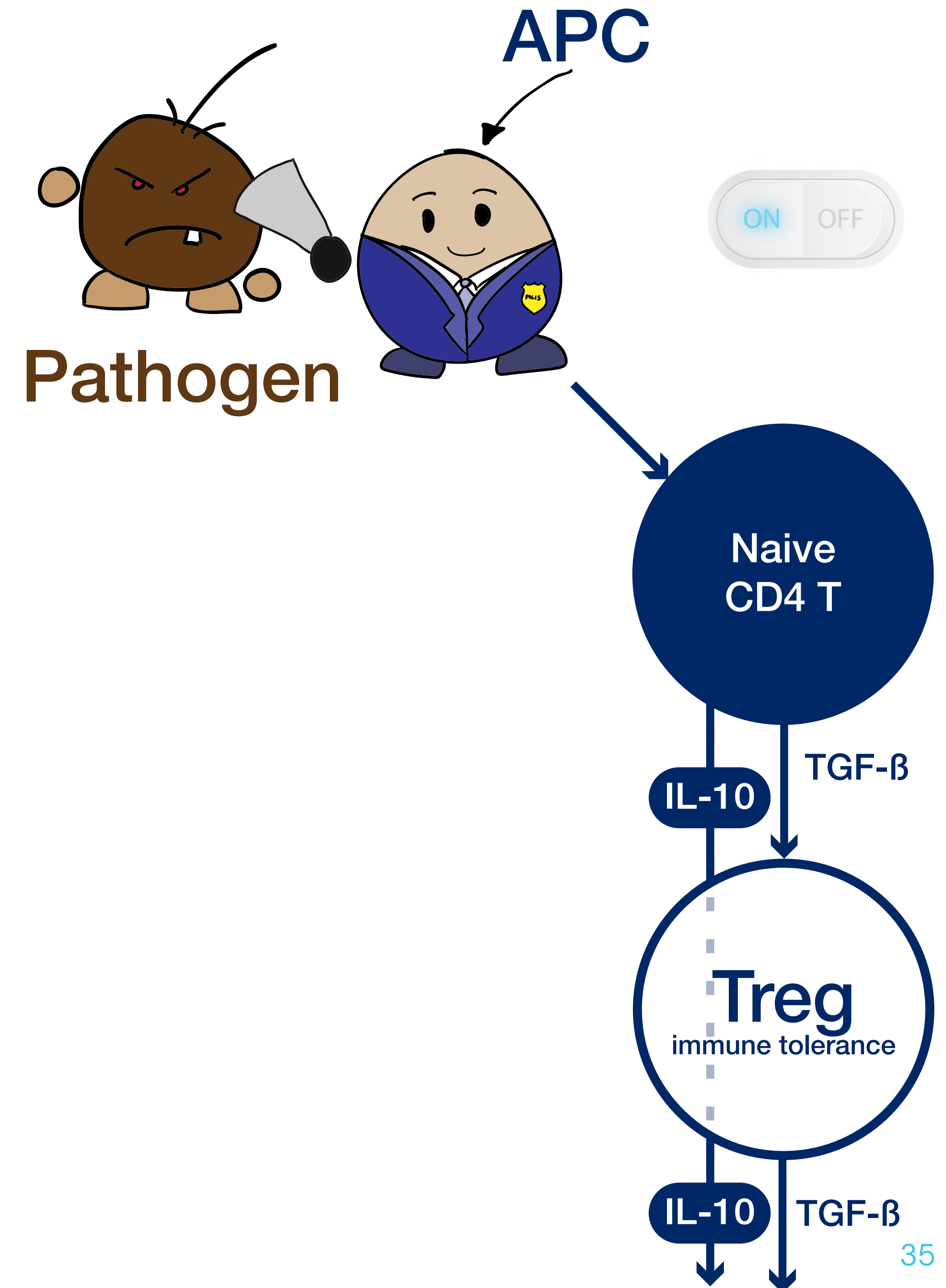


The most characteristic in auto-immunity is the lost in tolerance : poor T Reg activity

We improve tolerance if IL-10 goes up

- **Optimizing Vit D3** – individual
- **Butyrate coated:** metabolite produced by fermentation through anaerobic colon bacteria
Supplementation in coated form (Butyflam)
- **Transfer Factors**
Small proteins with RNA (nucleotide material)
Made by activated T-helper cells or pure amino acid extracts of colostrum

NK Cell Activity↑ + IL-10↑
- **Optimizing DHEA** – individual

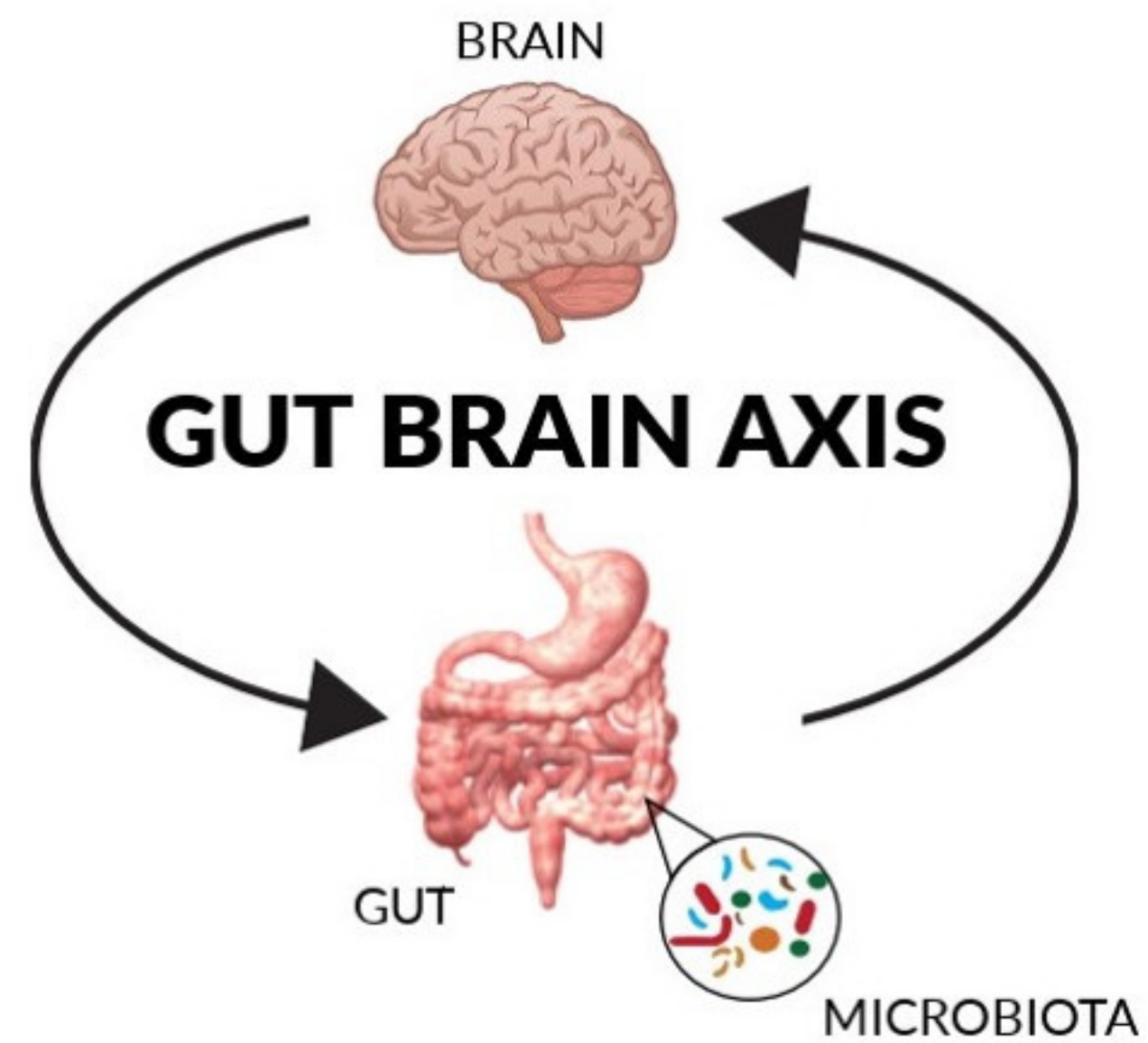


Auto-immunity = Functional Approach

- **Dietary advice**
- **Lifestyle advice**
- **Nutritional advice**

= keep the patient in remission

Control individual triggers to avoid auto-immune flare ups
Improve oral tolerance and self-tolerance



3. Neuroinflammation

Butyrate is one of the most important microbiota-generated metabolites involved in bidirectional crosstalk between the Gastro-Intestinal Tract & the Central Nervous System (CNS)

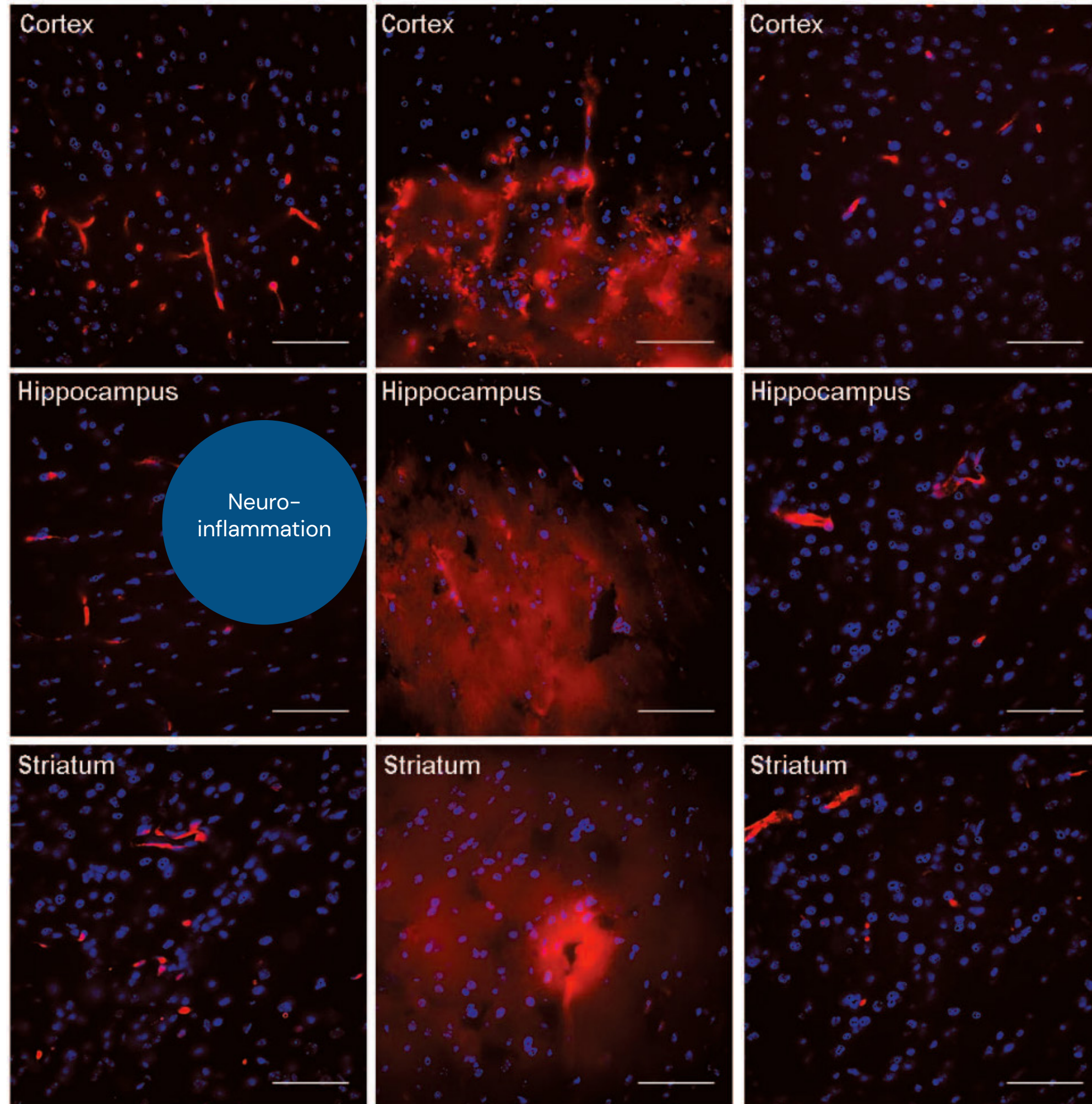
= The Gut-Brain axis

Butyrate and BBB permeability

Pathogen-free

Germ-free

SB



Germ-free mice: lack of normal gut bacteria



Increased BBB permeability

(compared to pathogen-free mice)

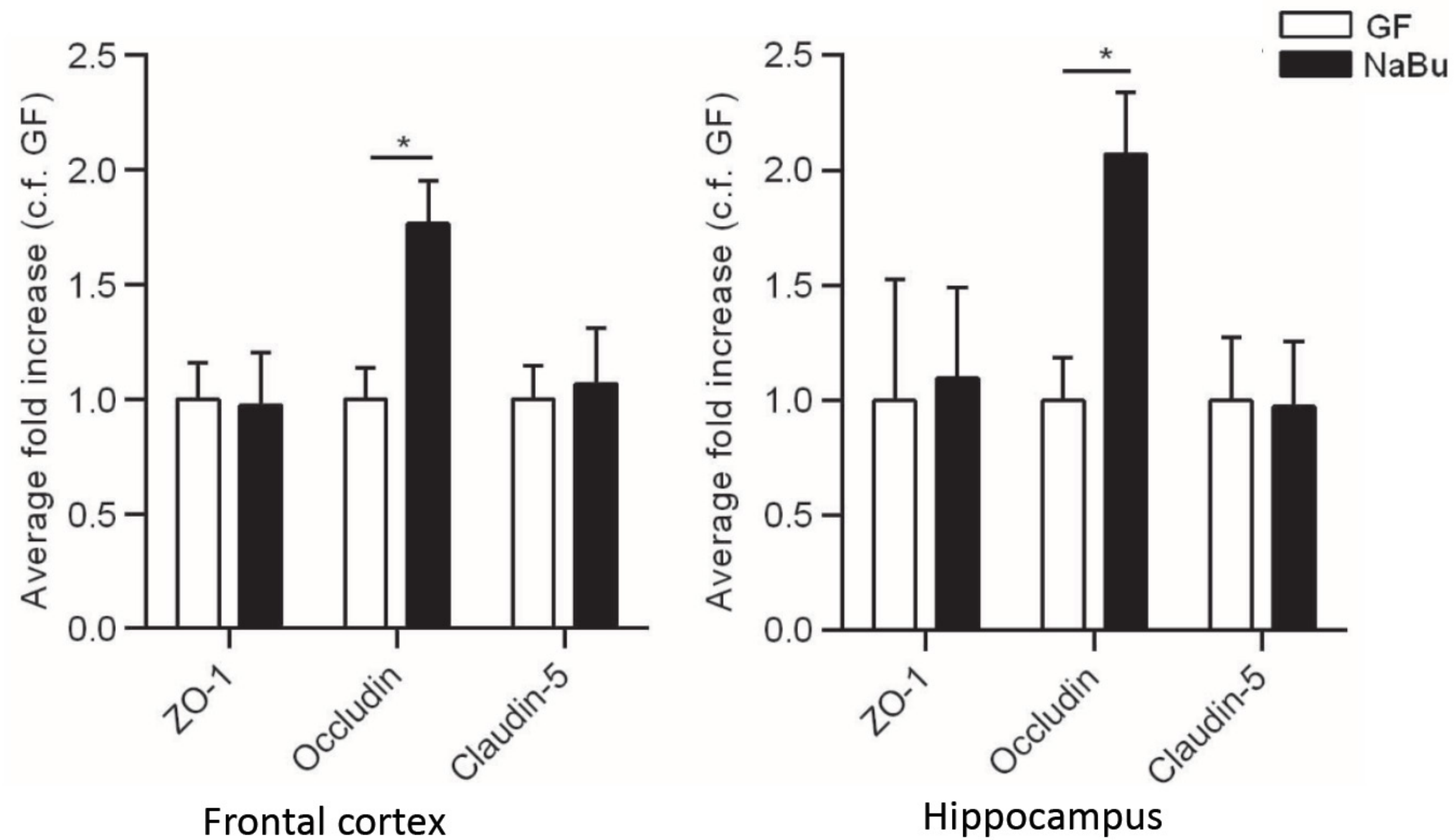
Sodium butyrate: 1g/kg, oral administration, 3 days



Restored BBB permeability

(equivalent to pathogen-free BBB)

Visualisation with Evans blue tracing



Butyrate gavaged mice (72h): increases the expression of **occludin**, compared to water gavaged mice



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