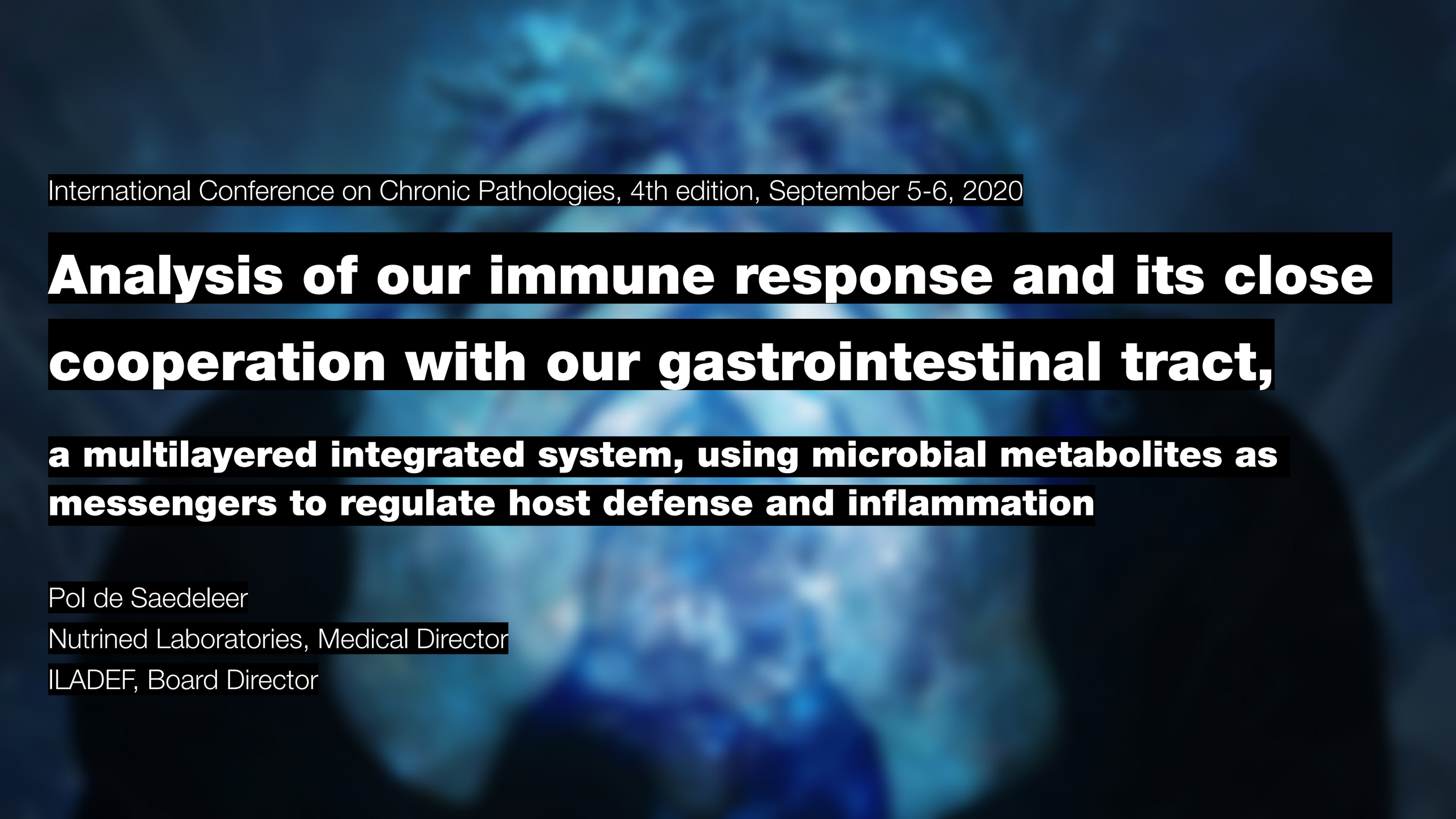


International Conference on Chronic Pathologies, 4th edition, September 5-6, 2020

Analysis of our immune response and its close cooperation with our gastrointestinal tract,

a multilayered integrated system, using microbial metabolites as messengers to regulate host defense and inflammation

Pol de Saedeleer
Nutrined Laboratories, Medical Director
ILADEF, Board Director

Our immunity is meant to protect us against all kind of threats

We are constantly surrounded by millions of bacteria, viruses and other germs.

Our immune system is our defense against disease -causing agents.

Immunity = different barriers

Skin & epithelial surfaces

Cavities such as mouth and nose but also our gastrointestinal tract are lined up with mucous membranes & covered with mucus

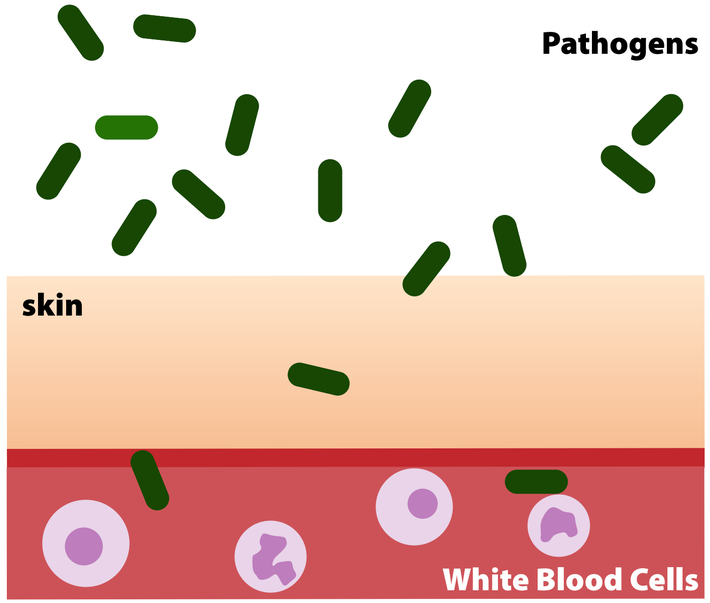
Fluids like gastric acid or saliva

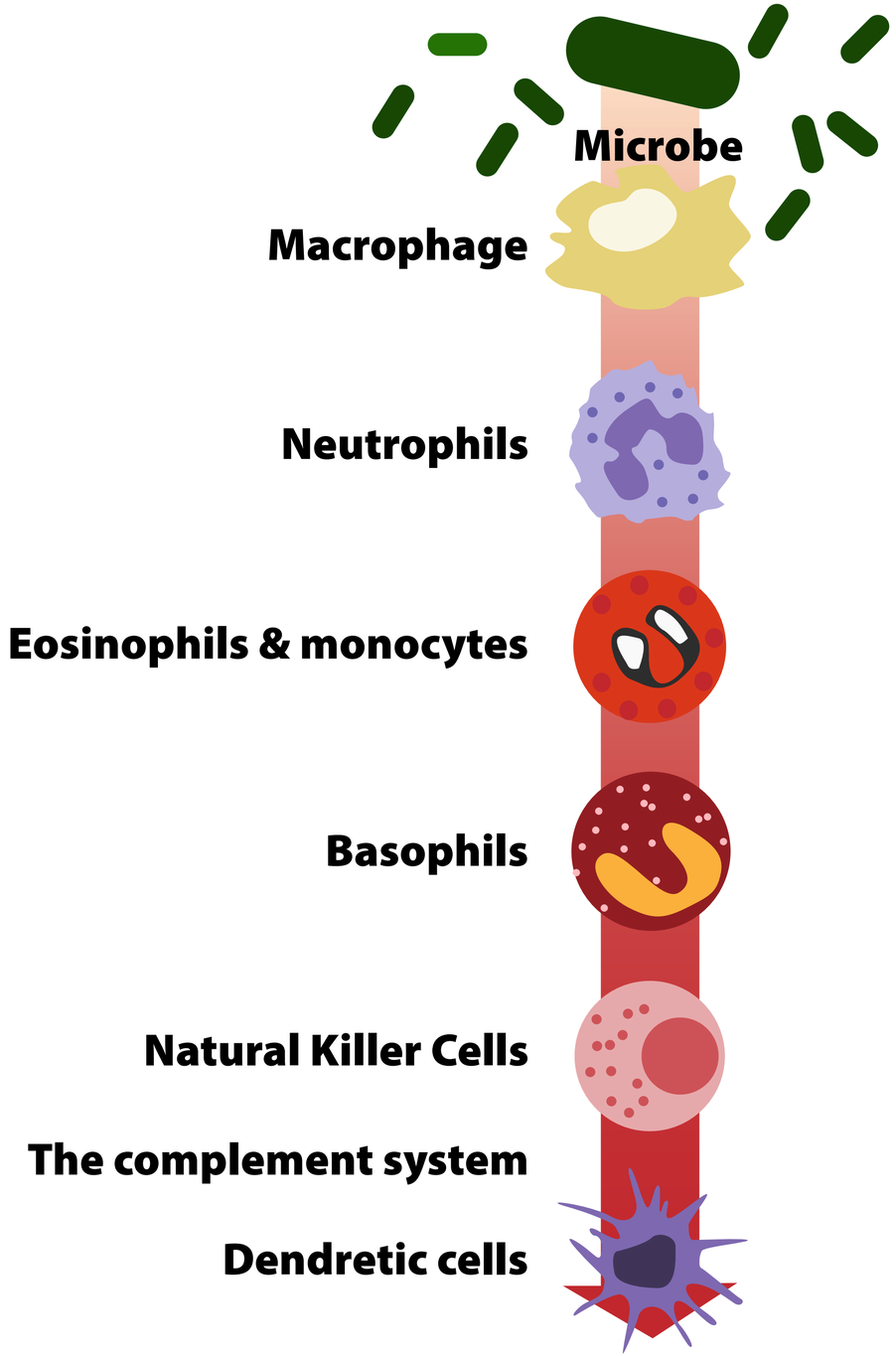
The innate immunity
= the part of your immune system you were born with

White blood cells travelling in your bloodstream
Immediate reaction, no memory

The adaptive immunity
= the immunity that you develop when you grow

B-and T -lymphocytes in lymph and lymphnodes
Delayed reaction, more sophisticated and specific, with a memory

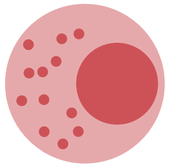




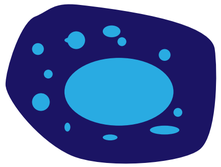
Innate Immunity

Macrophage

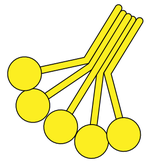

Dendritic Cell

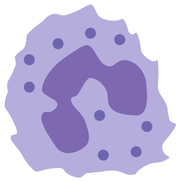

NK cell

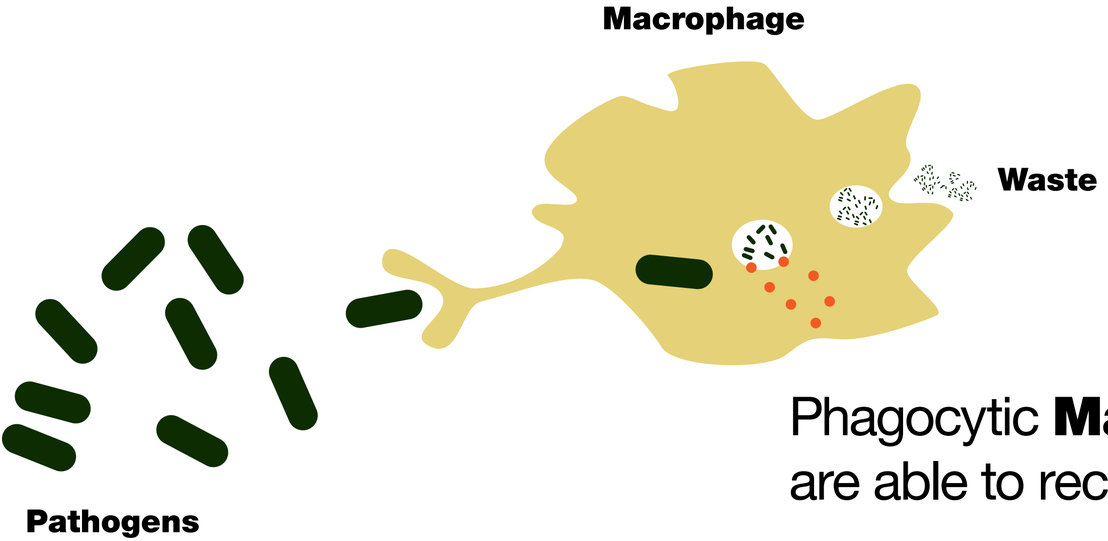

Basophil


Mast Cell


Eosinophil


Complement protein


Neutrophil



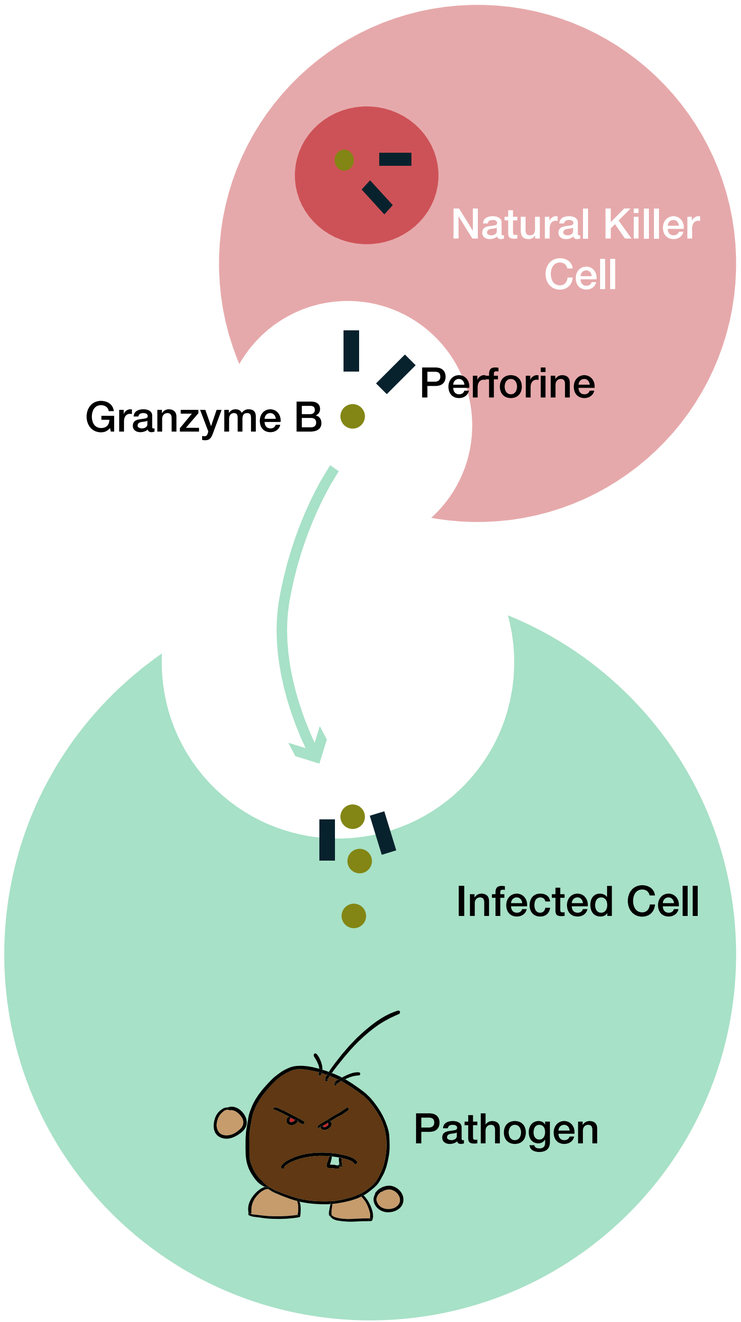
Phagocytic **Macrophages**, by means of surface receptors, are able to recognize and bind parts of the bacterial surfaces

Bacteria bind to these receptors and trigger the macrophage to **engulf** the bacterium and induce the secretion of biologically active molecules

Once engulfed the microbes are killed by digestive enzymes, generated ROS, antimicrobial peptides = defensins and cathelicidins

Macrophages release chemokines that attract cells with chemokine receptors such as **neutrophils and monocytes** from the bloodstream

Macrophages release cytokines
The release of **chemokines and cytokines initiate the process of inflammation**

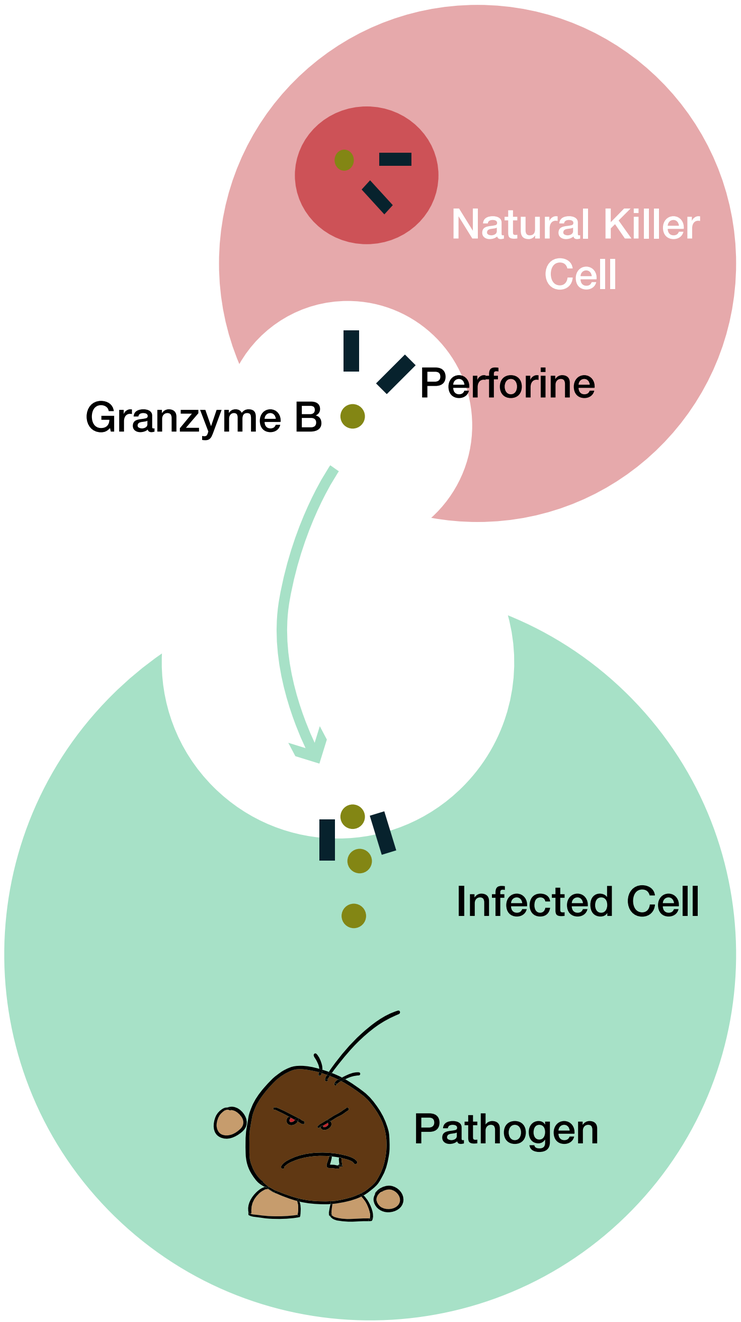


Natural Killer Cells

NK Cells kill viruses and cancer cells

Stimulation of NK Cells
NK cells have inhibitory and activating receptors
NK Cells respond to triggers on the surface of target Cells

NK Cells need to pro-inflammatory cytokines to get activated:
IL-12, IL-15, IL-2, IL-18, Interferons



How do they attack?

NK Cells release small granules containing enzymes, known as granzymes

Secretion of immunoregulatory cytokines such as IFN-γ

Cytotoxic activity by releasing perforin and granzymes

NK Cells secrete immunoregulatory cytokines like IFN-γ & TNF-α

Cytotoxic activity by releasing perforin and granzymes

NK Cells respond rapidly

Patient deficient in NK Cells are highly susceptible to viral infections

NK-cells affected by ROS lost the adherence to target cells in both in vitro and in vivo

ROS may change the surface of NK-cells, resulting in an inability of adhesion to target cells

Susceptibility of Natural Killer (NK) Cells to Reactive Oxygen Species (ROS) and Their Restoration by the Mimics of Superoxide Dismutase (SOD)

Kunie Nakamura[1] and Ken-ichi Matsunaga[2]
[1]Molecular Biology Laboratory, Department of Biochemistry, Kitasato University School of Medicine, Kanagawa, Japan; [2]Biomedical Research Laboratories, Kureha Chemical Industry, Co. Ltd., Tokyo, Japan.

Natural killer (NK) cells are susceptible to reactive oxygen species (ROS), and lose the activity by the effects of ROS. Cancer bearing hosts usually suffer from oxidative stress (OS), and the NK-activity decreases to a significantly lower level than normal controls. Superoxide dismutase (SOD)-mimicking substances, such as protein-bound polysaccharide of Coriolus versicolor (Fr) QUEL (PSK) and iron-chelating chlorin e6-Na (FeCNa), can restore the NK-activity of cancer bearing hosts, when collaborating with catalase. Incorporation of ^{3}H-thymidine by ROS-treated NK-cells is not affected, indicating that these cells are still active in the nucleic acid metabolism. Intraperitoneal administration of anti-Asialo GM1 antibody extinguished the NK-activity. NK-cells affected by ROS lost the adherence to target cancer cells in both in vitro and in vivo. ROS may change the surface charge of NK-cells to anionic, resulting in an inability of adhesion to target cancer cells which usually show the negative surface charge.

NEW RESEARCH ON LIPOSOMAL GLUTATHIONE

Clinical research presented at the 2015 ILADS medical conference demonstrated red blood cell absorption, increased natural killer cell function & reduced oxidative stress.

This study, conducted at Penn State University, measured the efficacy of Researched Nutritionals' Tri-Fortify™ liposomal glutathione. The product is formulated to promote healthy intracellular glutathione levels and natural killer cell function.

Healthy patients with glutathione levels at the low end of the normal range were included in the study. The patients were divided into two groups, one taking one serving (450mg) per day and the other taking two servings (900mg) per day. Both groups performed fairly close to one another so the results shown below are the combined impact, with a p-value of <0.05.

BACKGROUND

Glutathione is a tri-peptide, composed of the three amino acids: L-glutamic acid, L-cysteine and L-glycine. Healthy glutathione level plays a significant role in promoting normal:

- Detoxification
- Immune Response
- Antioxidant Action

Glutathione is required to maintain the normal function of the liver and to protect DNA, proteins & mitochondria against oxidative stress, while supporting NK cell function & lymphocyte (white blood cell) proliferation.

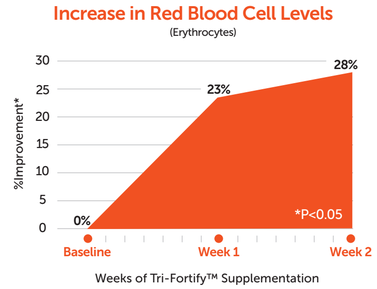
Researchers and clinicians view red blood cell absorption as essential for the body to achieve the benefits of exogenous glutathione supplementation.

RESEARCH RESULTS

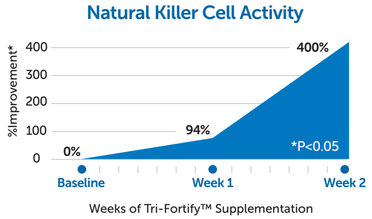
After two weeks of daily oral liposomal glutathione supplementation:

- Red blood cell levels (erythrocytes) increased 28% over the baseline.
- Natural Killer Cell function increased by 400% over the baseline.
- Oxidative stress, (as measured by lipid peroxidation) decreased by 25%

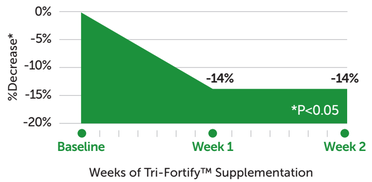
GLUTATHIONE LEVELS

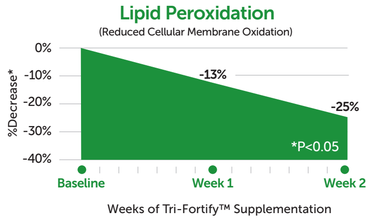


IMMUNE FUNCTION



OXIDATIVE STRESS MARKERS

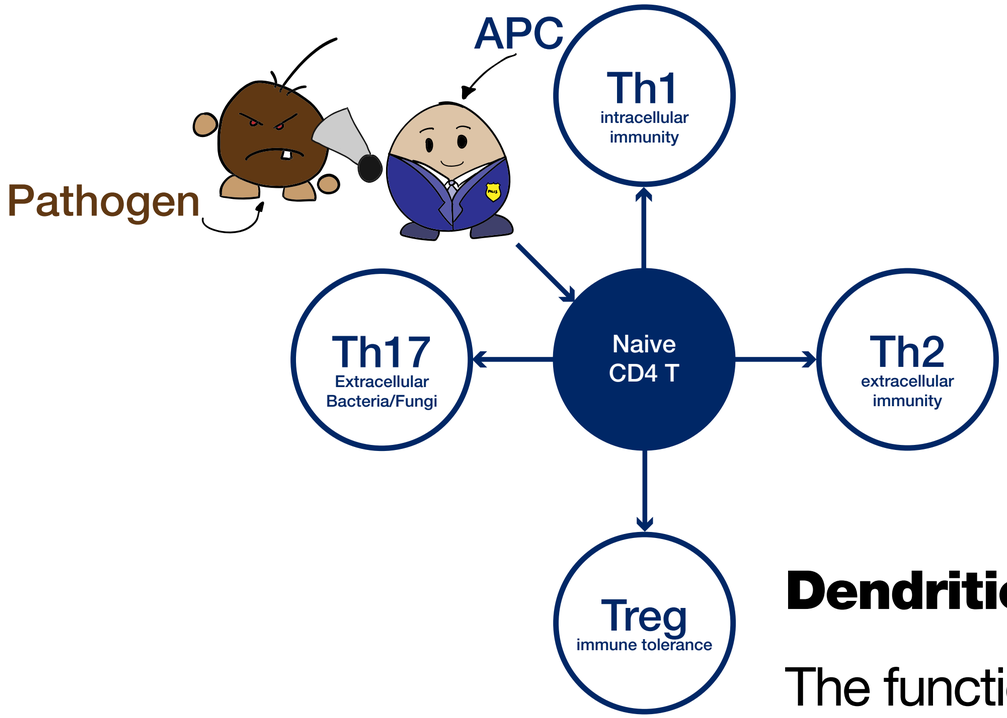




Published study shows Liposomal Glutathione (Trifortify) is increasing Natural Killer Cell Activity by reducing oxidative stress

Sinha, Raghu, et al. "Oral supplementation with liposomal glutathione elevates body stores of glutathione and markers of immune function." European journal of clinical nutrition 72.1 (2018): 105-111.

Daily dose = 1 teaspoon/day



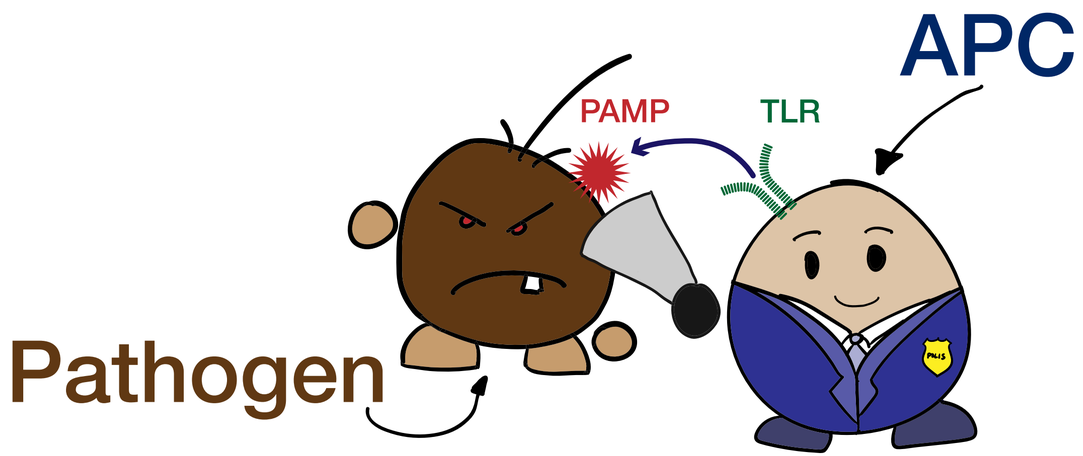
Dendritic cells initiate the adaptive immune system

The function of dendritic cells is not to primarily to destroy pathogens but to carry pathogens and to activate Lymphocytes

Dendritic Cells → **APC**

APC migrate through the lymph to the regional lymph nodes where they will interact with the naïve lymphocytes

There is a delay of 4-7 days until the adaptive immunity gets organized
During this period the innate immunity has a critical role in controlling infections



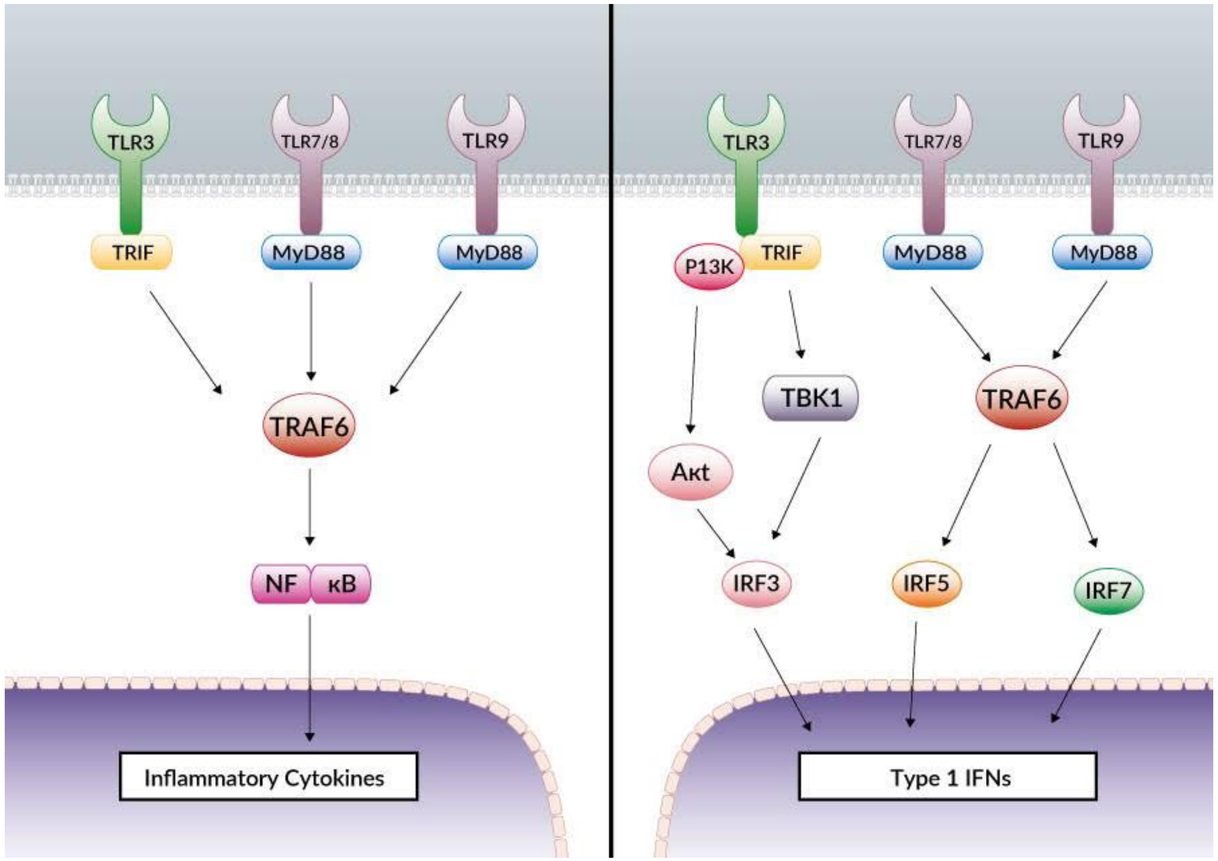
Antigen Presenting Cells with Pattern Recognition Receptors (PRR) recognize Pathogen Associated Molecular Patterns (PAMPs) on microbes

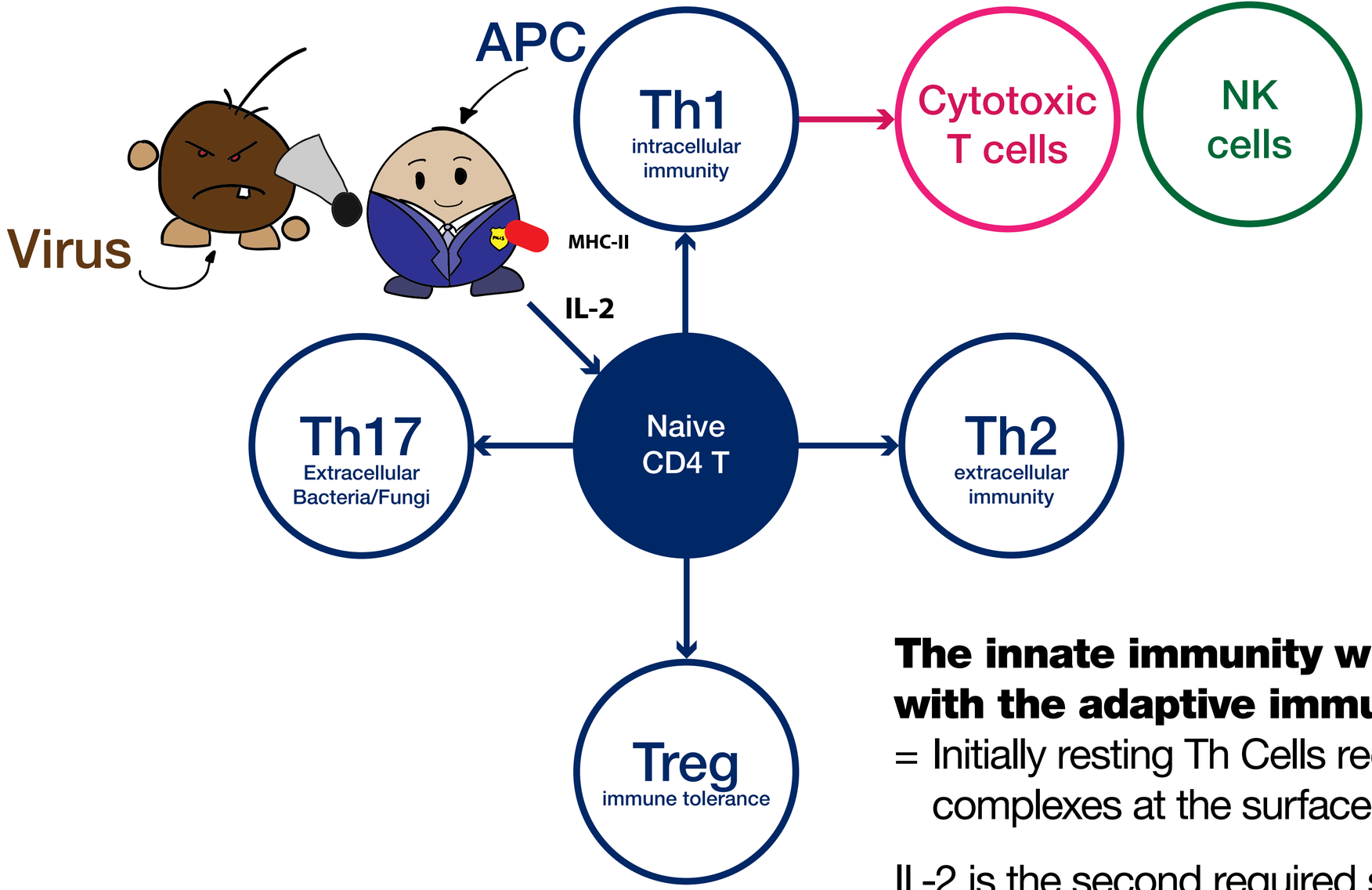
PRRs are often Toll-like receptors (TLR's)

TLR3 recognizes double stranded RNA (ds RNA)

TLR 7 & TLR 8 recognize single stranded RNA (ss RNA)

TLR9 recognizes DNA viruses





The innate immunity will now communicate with the adaptive immunity:

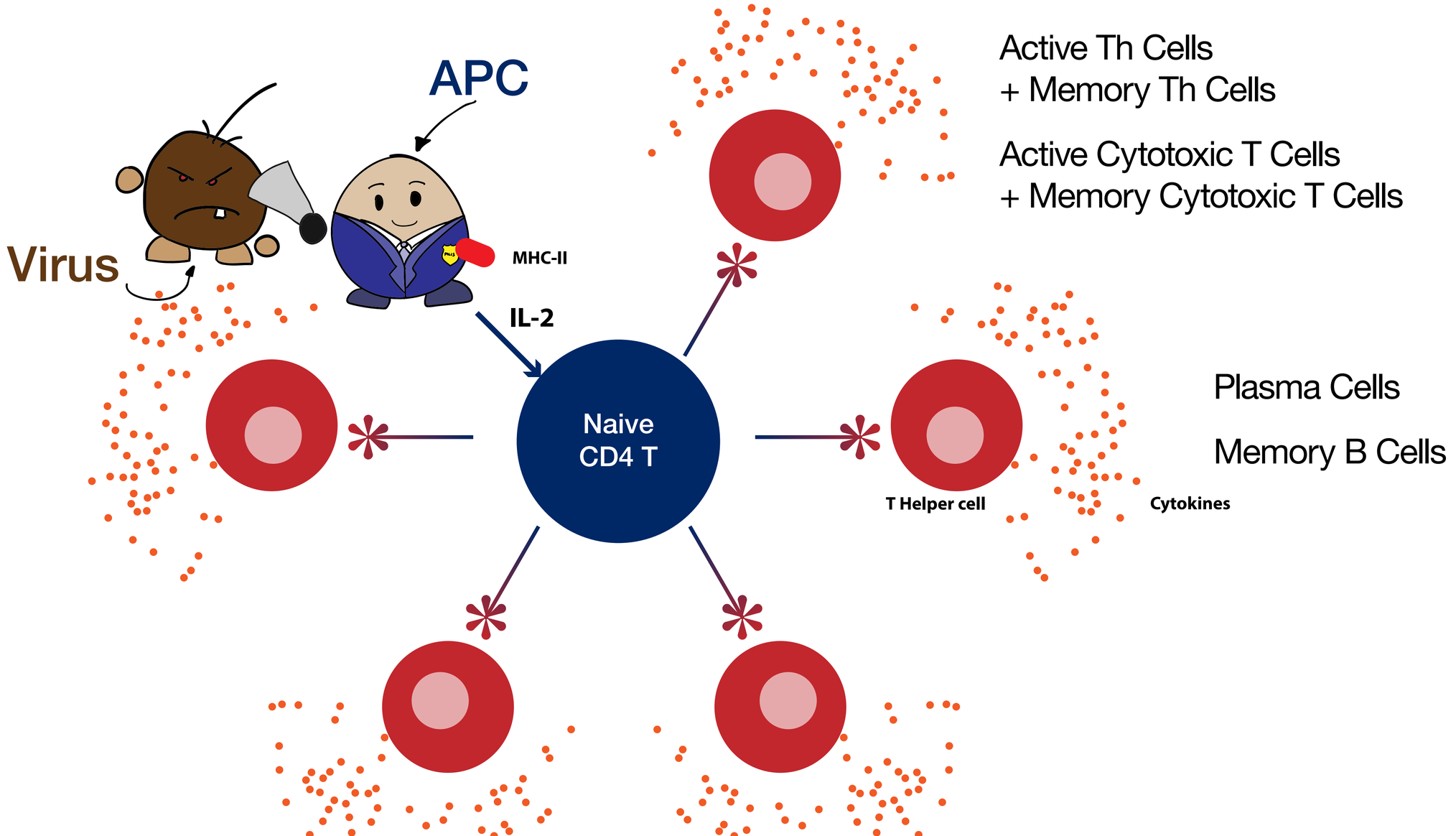
= Initially resting Th Cells recognize antigen-MHC-II complexes at the surface of APC

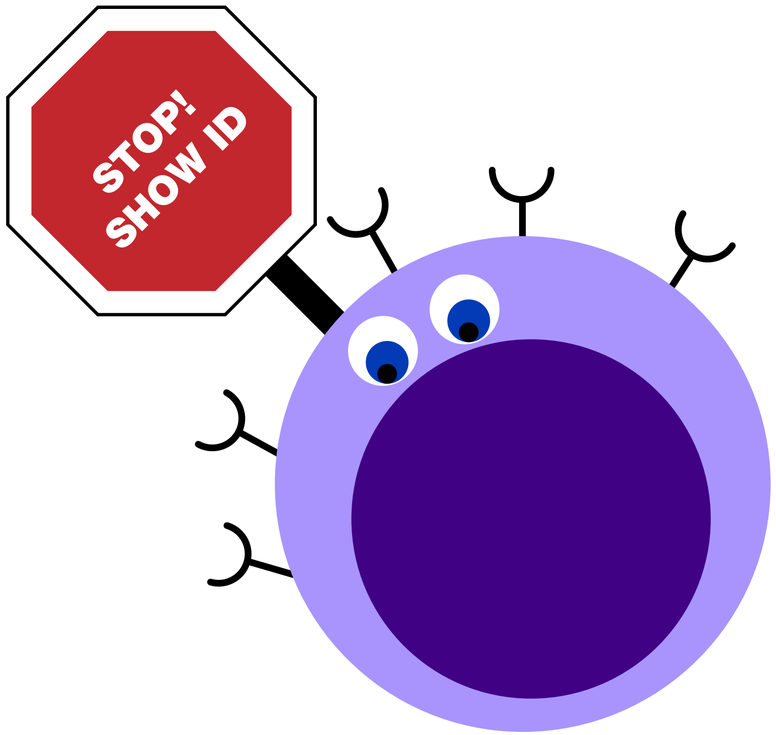
IL-2 is the second required signal for activation

IL-2 is required for virtually all immune responses, and is the prime trigger for T-cell proliferation

A variety of cytokines is secreted that will induce the adaptive immune system

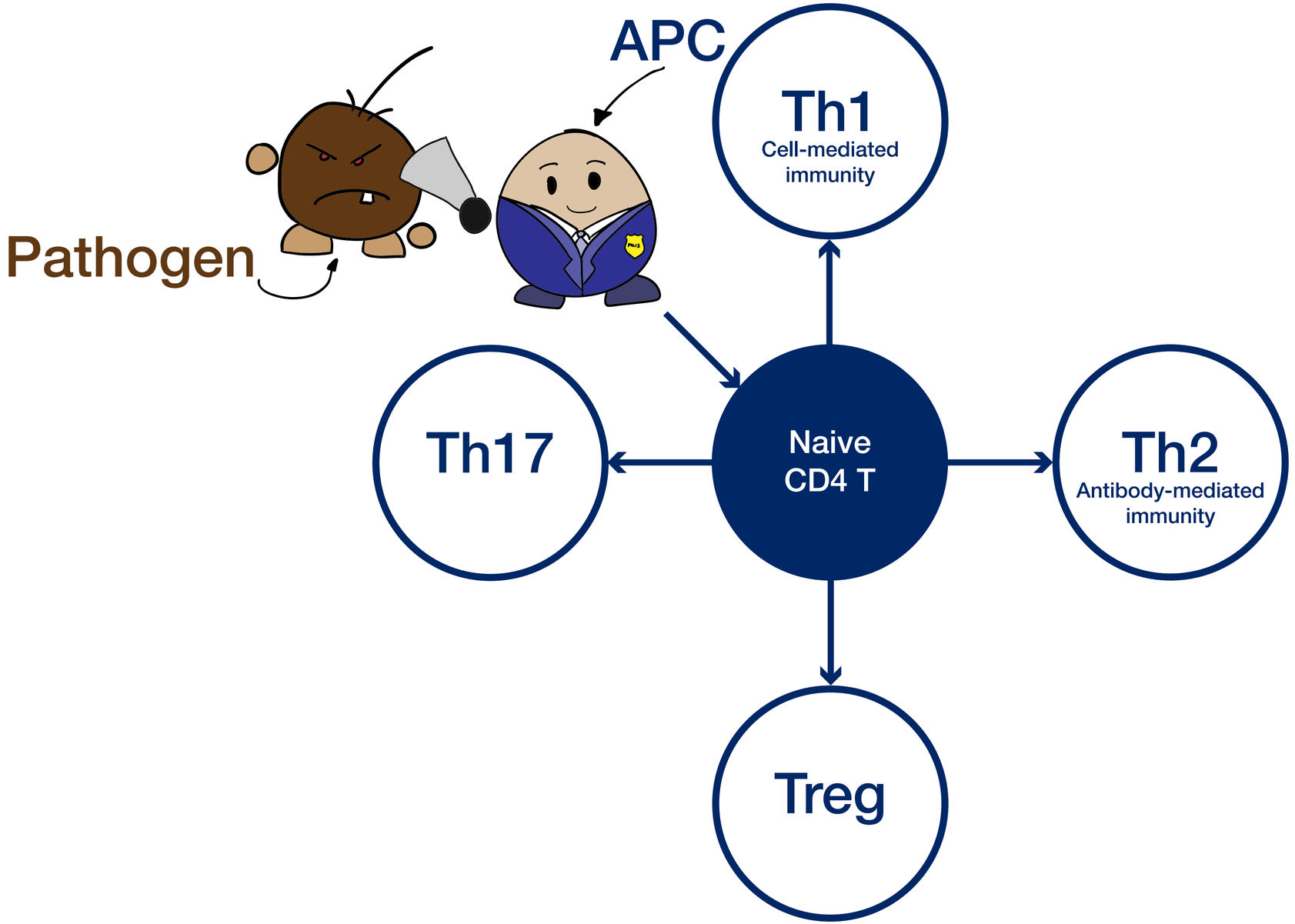
The activated T helper Cells will now help and assist the other lymphocytes



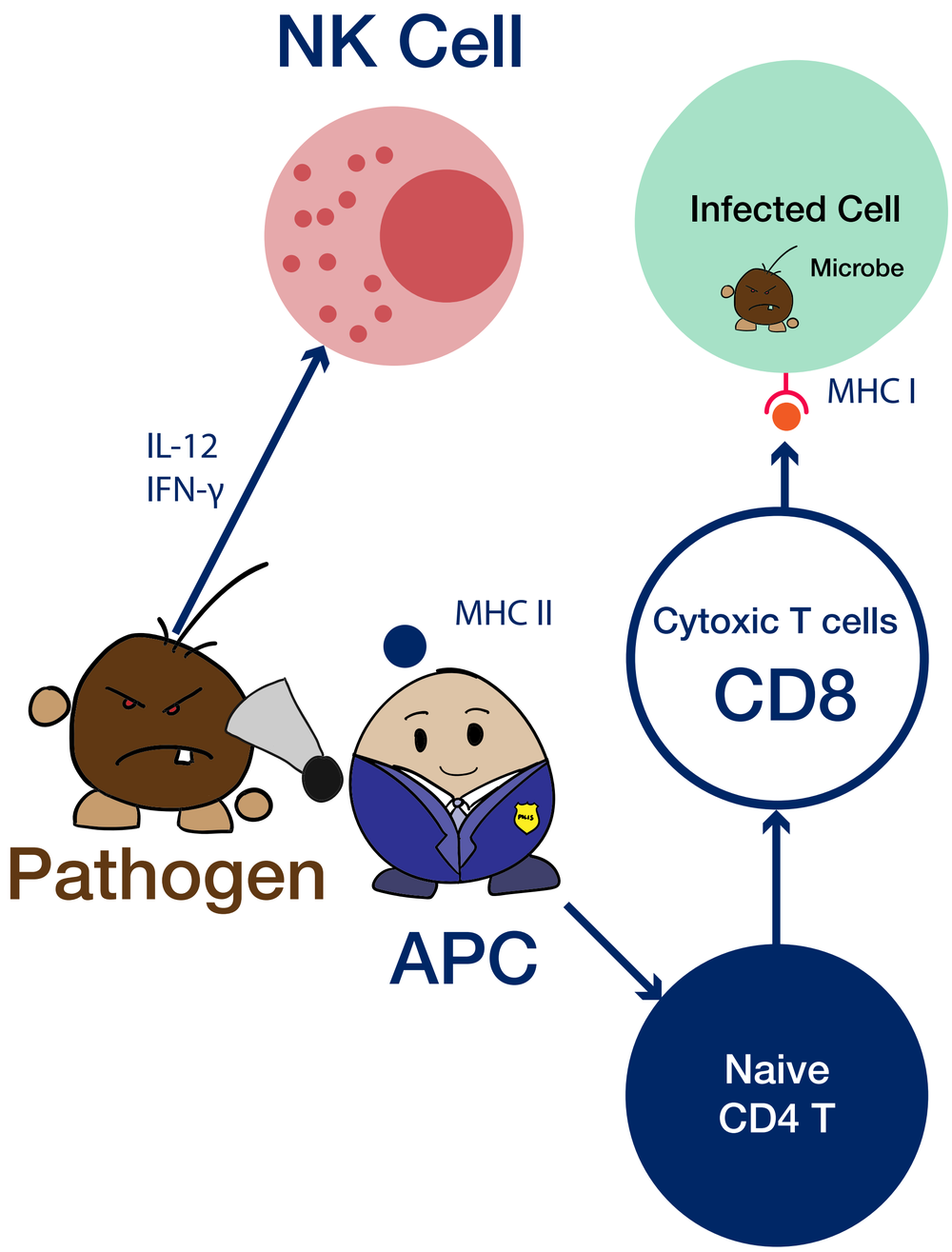


Memory Cells have long life span, often for decades

Memory Cells are able to proliferate and differentiate into Th Cells or Cytotoxic T cells , Plasma Cells or B Cells within hours



CD4 will determine how we will respond to the attack

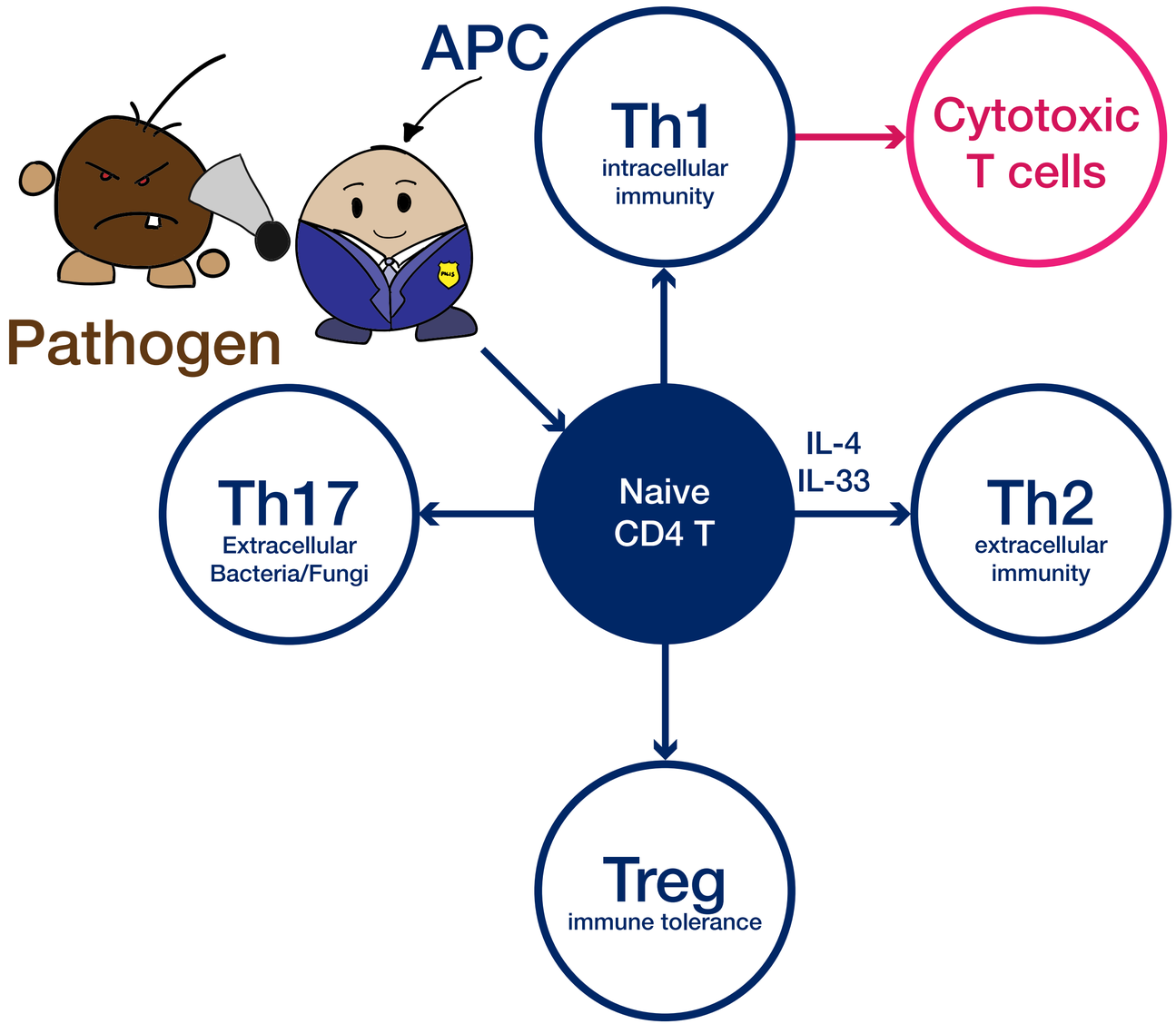


Cell Mediated Immunity

The immune response we manifest in intracellular infections

The role of NK Cells is similar to CD8 Cytoxic Cells

- MHC1 is expressed by all nucleated cell
- MHC2 complexes are only expressed by APC
  often used as a marker for microglial activity
- MHC1 enables antigen presentation to CD8
- MHC2 enables antigen presentation to CD4



Antibody mediated immunity Th2

Mainly works against extracellular pathogens

B cells

- Plasma Cells secrete antibodies
- Antibodies neutralize the antigen
- Antibodies activate the Complement system or Phagocytic Cells

Memory B cells

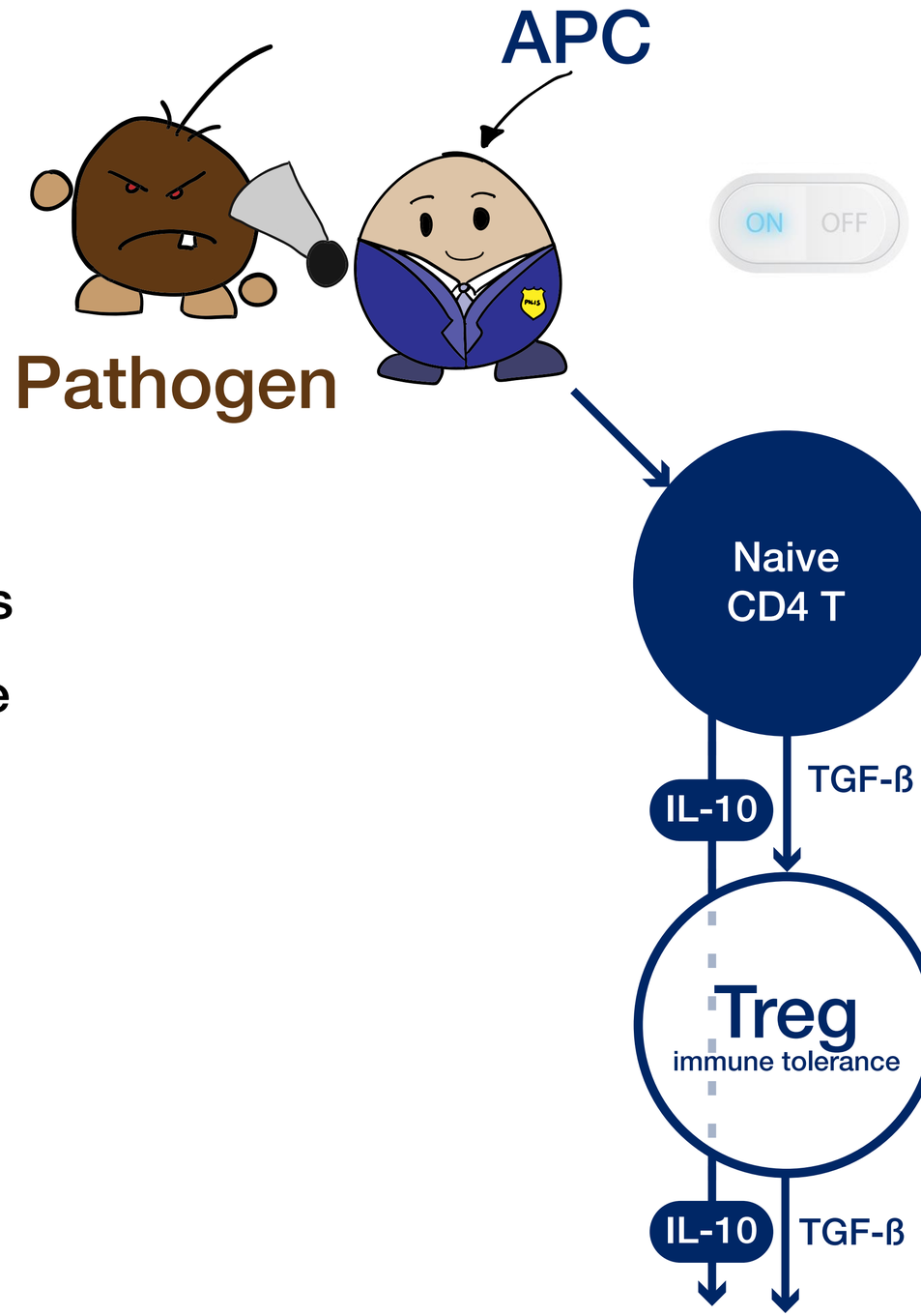
Do not secrete antibodies but instead quickly proliferate and differentiate into more plasma cells and memory B cells if the antigen reappears in the future

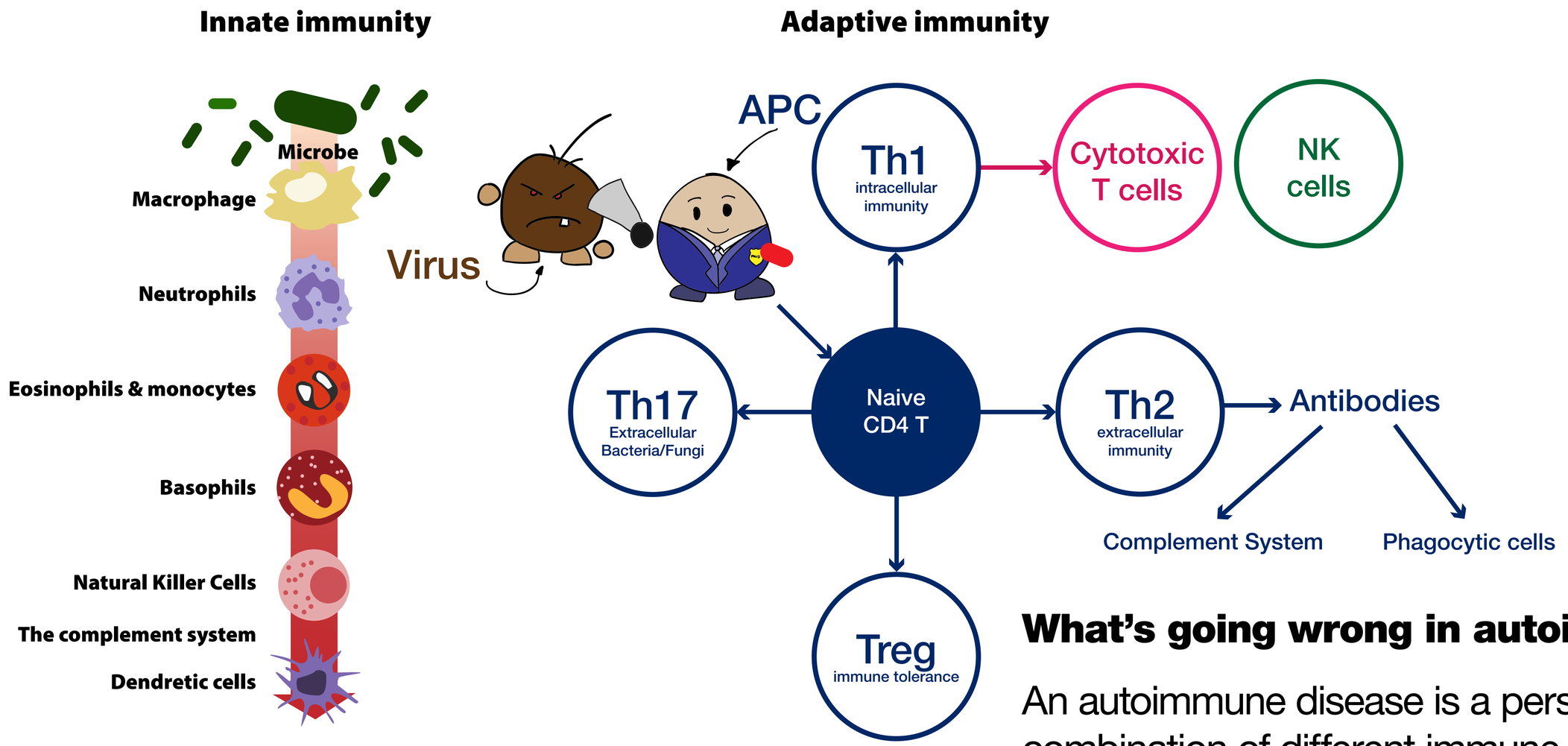
Regulatory T Cells (T reg's)

Decide about tolerance = also called suppressor T cells

Regulatory T Cells prevent excessive immune response

- Auto-immunity
- Excessive inflammation

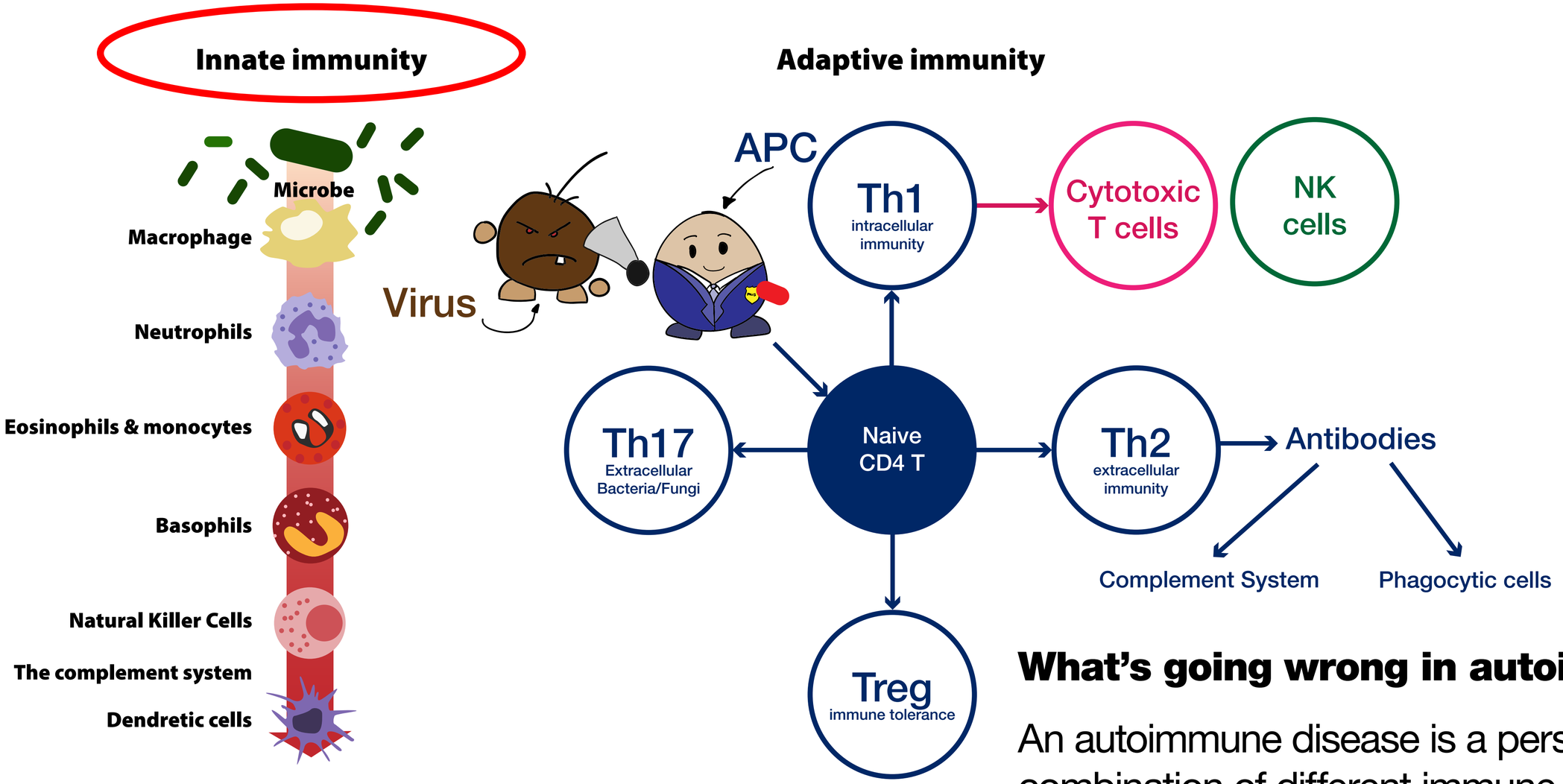




What's going wrong in autoimmunity?

An autoimmune disease is a personalized combination of different immune dysfunction patterns

The most characteristic is the loss of self-tolerance, T reg



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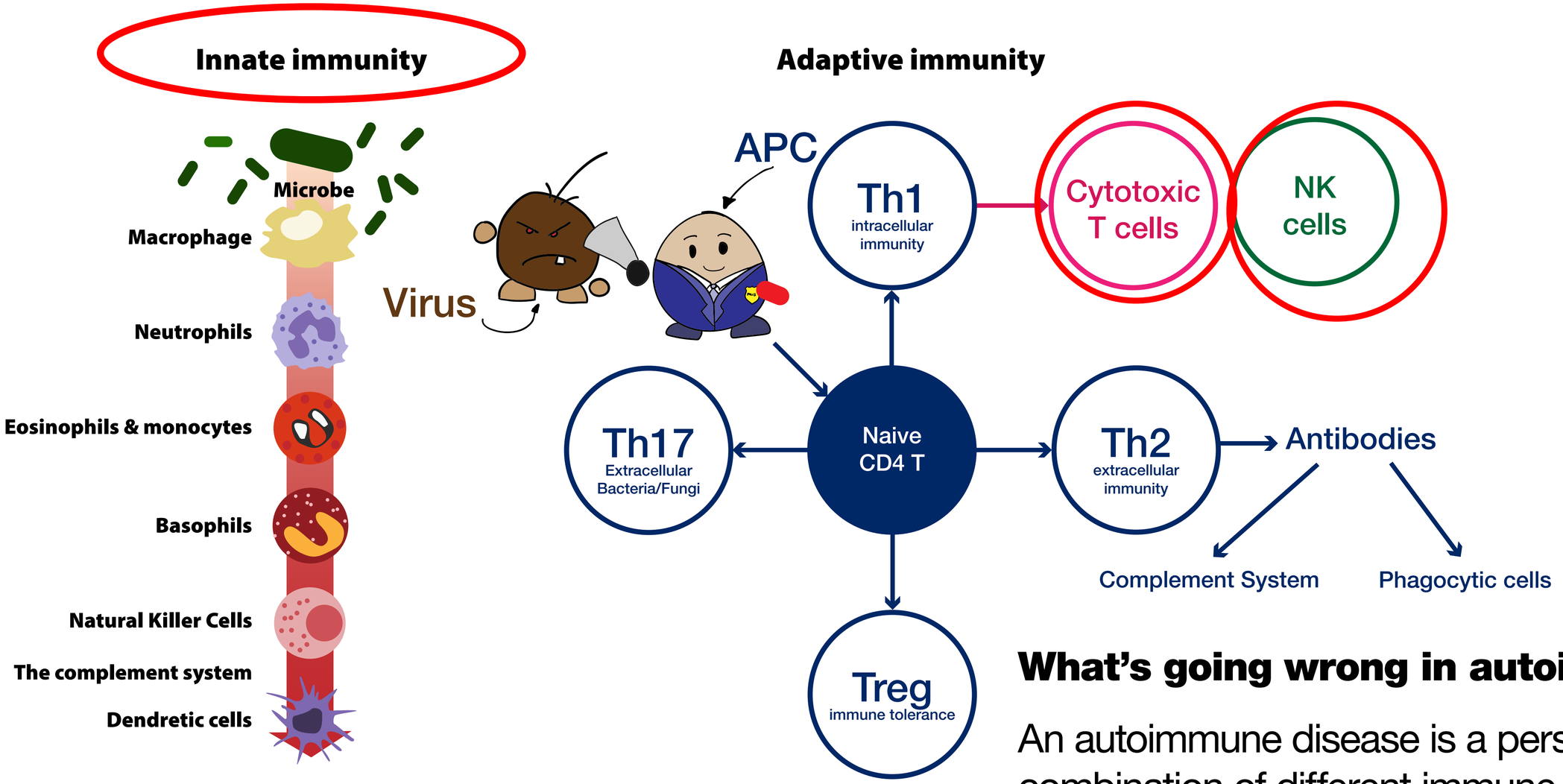

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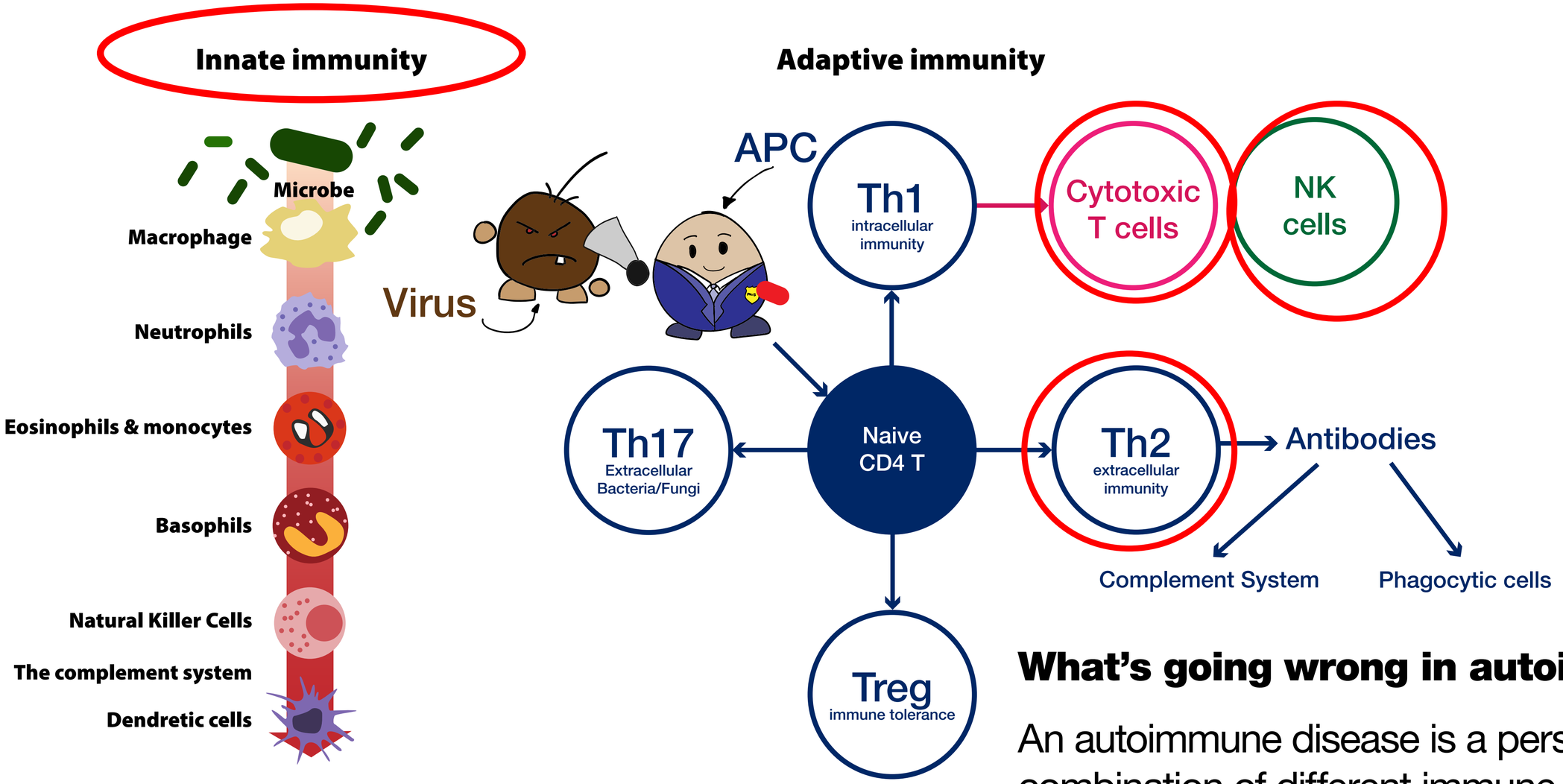

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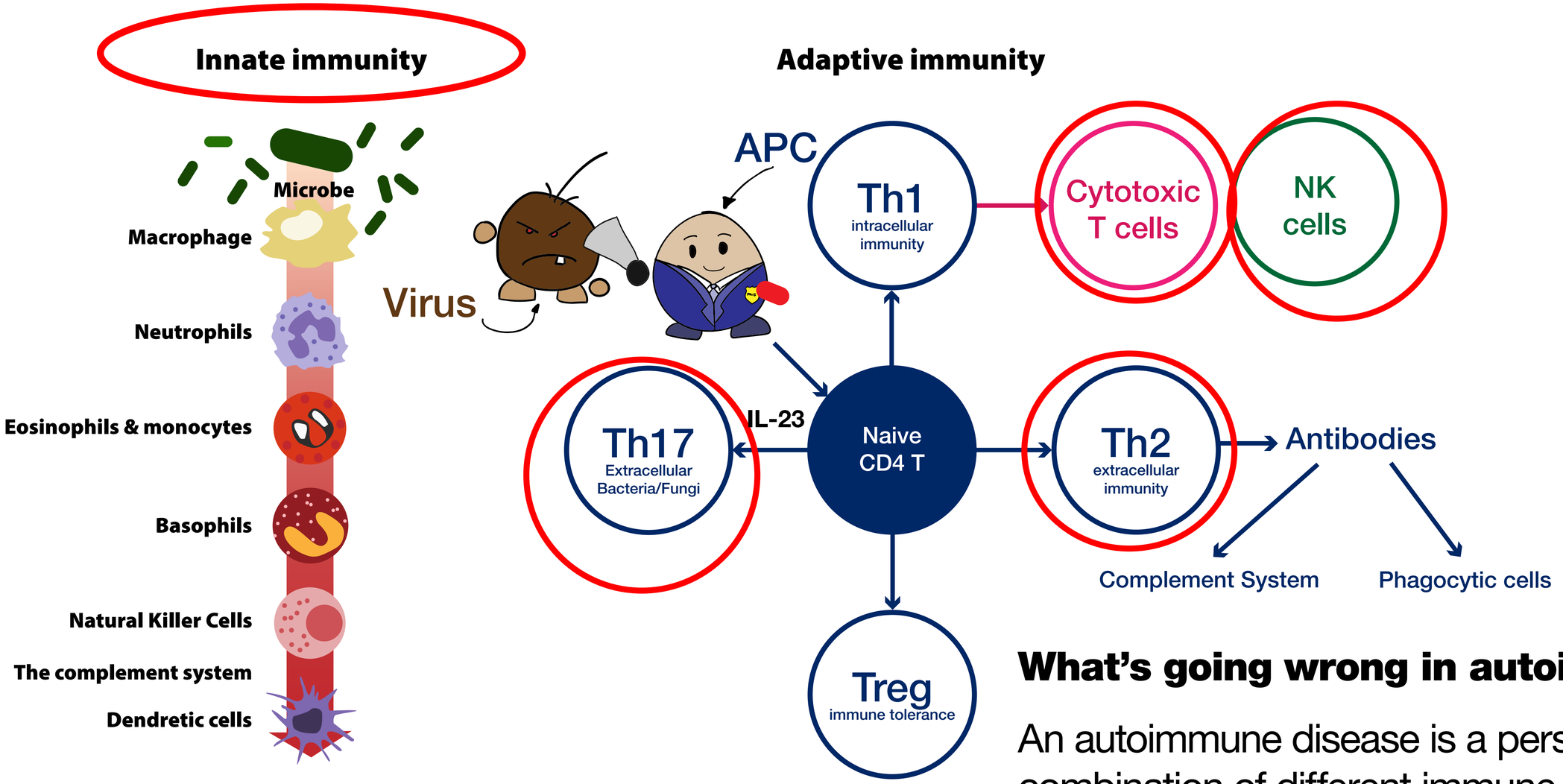

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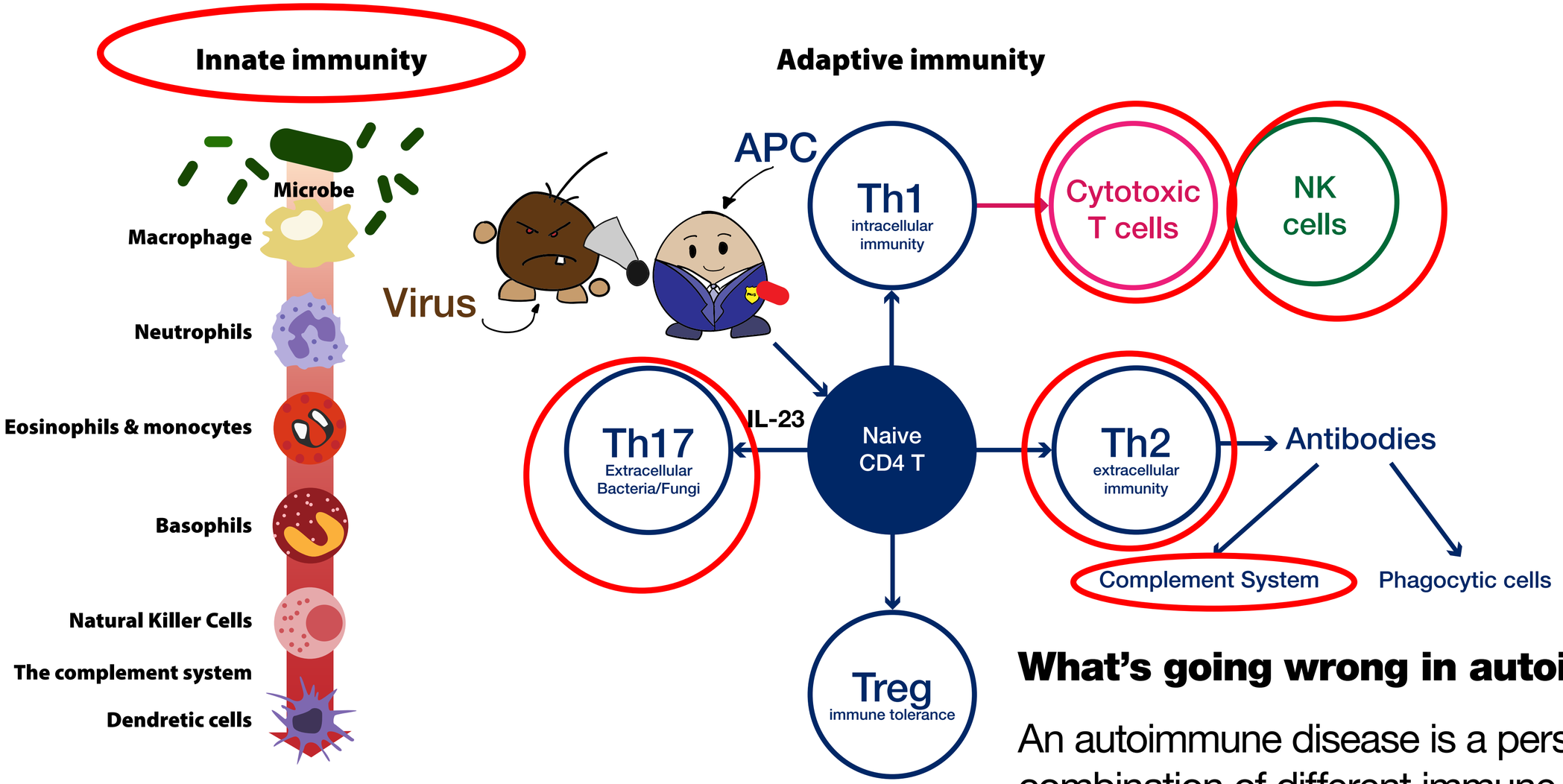

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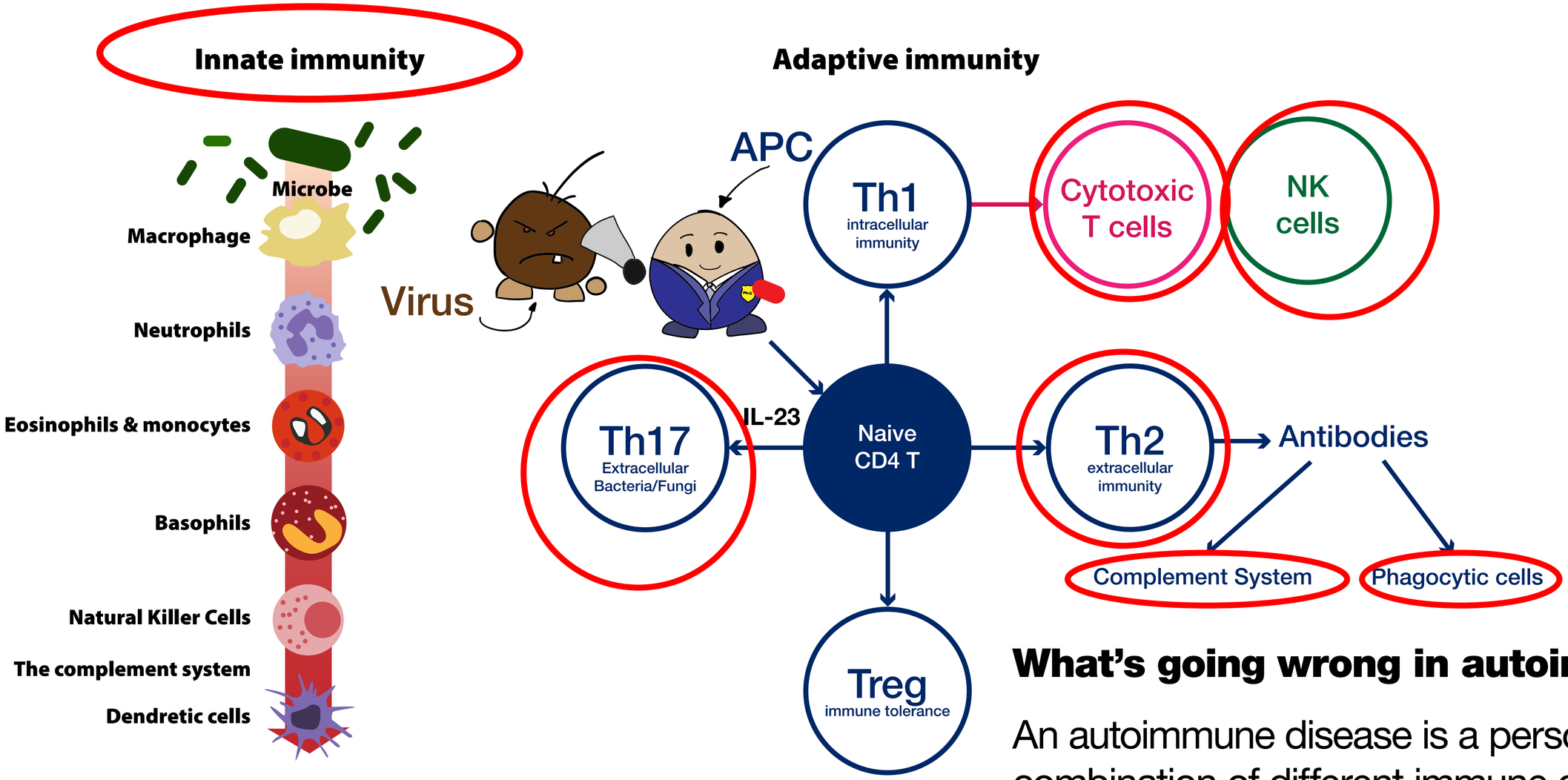

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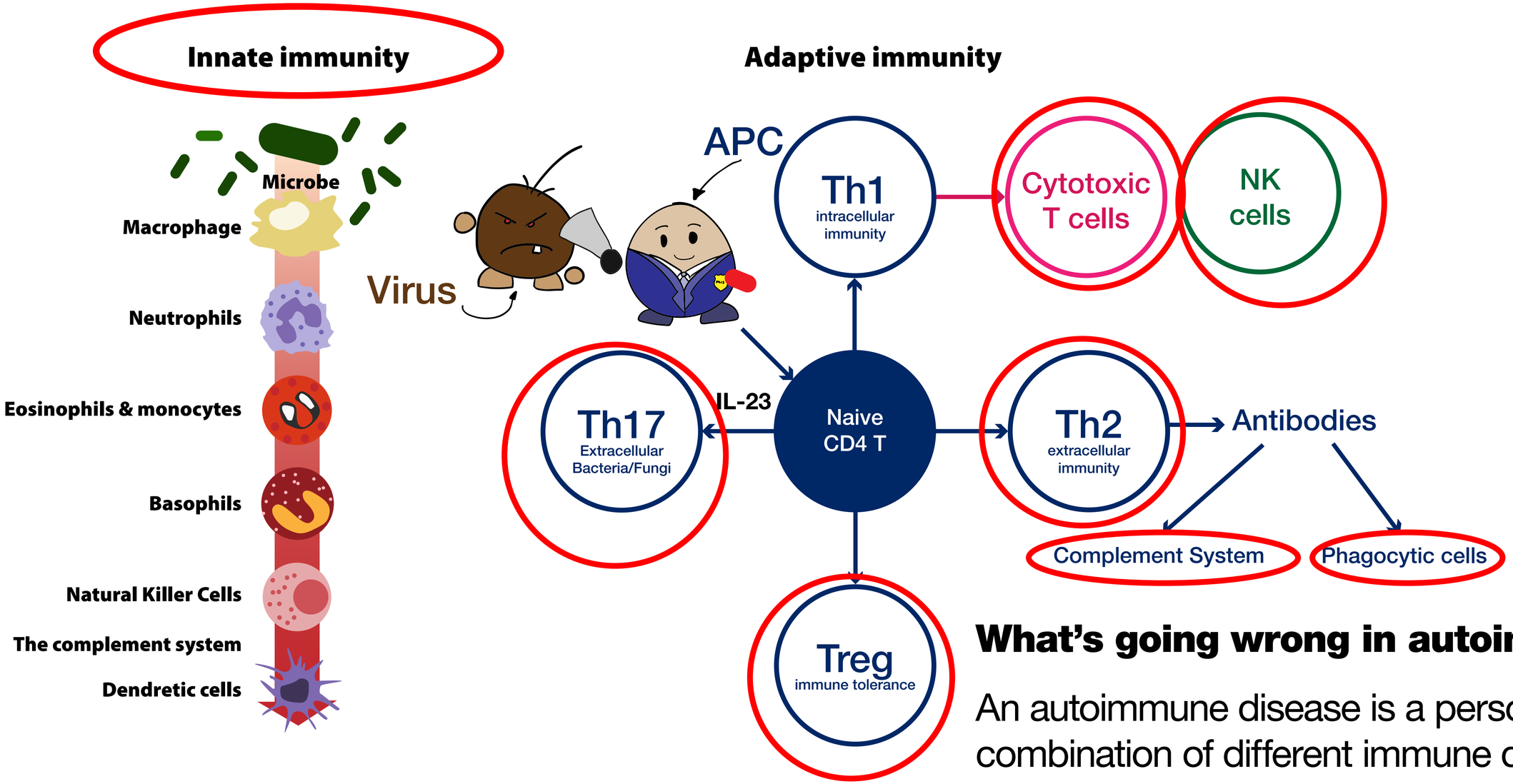

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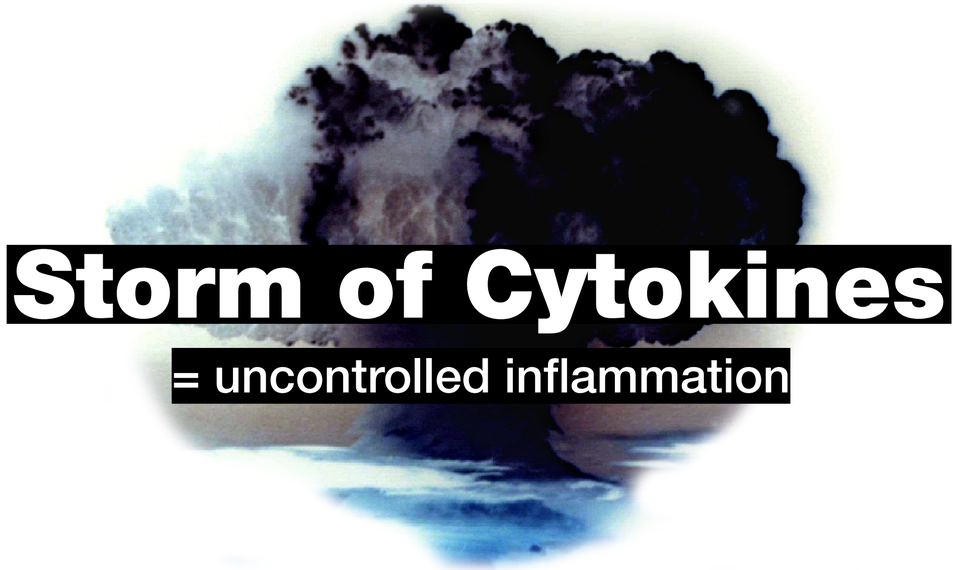


Storm of Cytokines

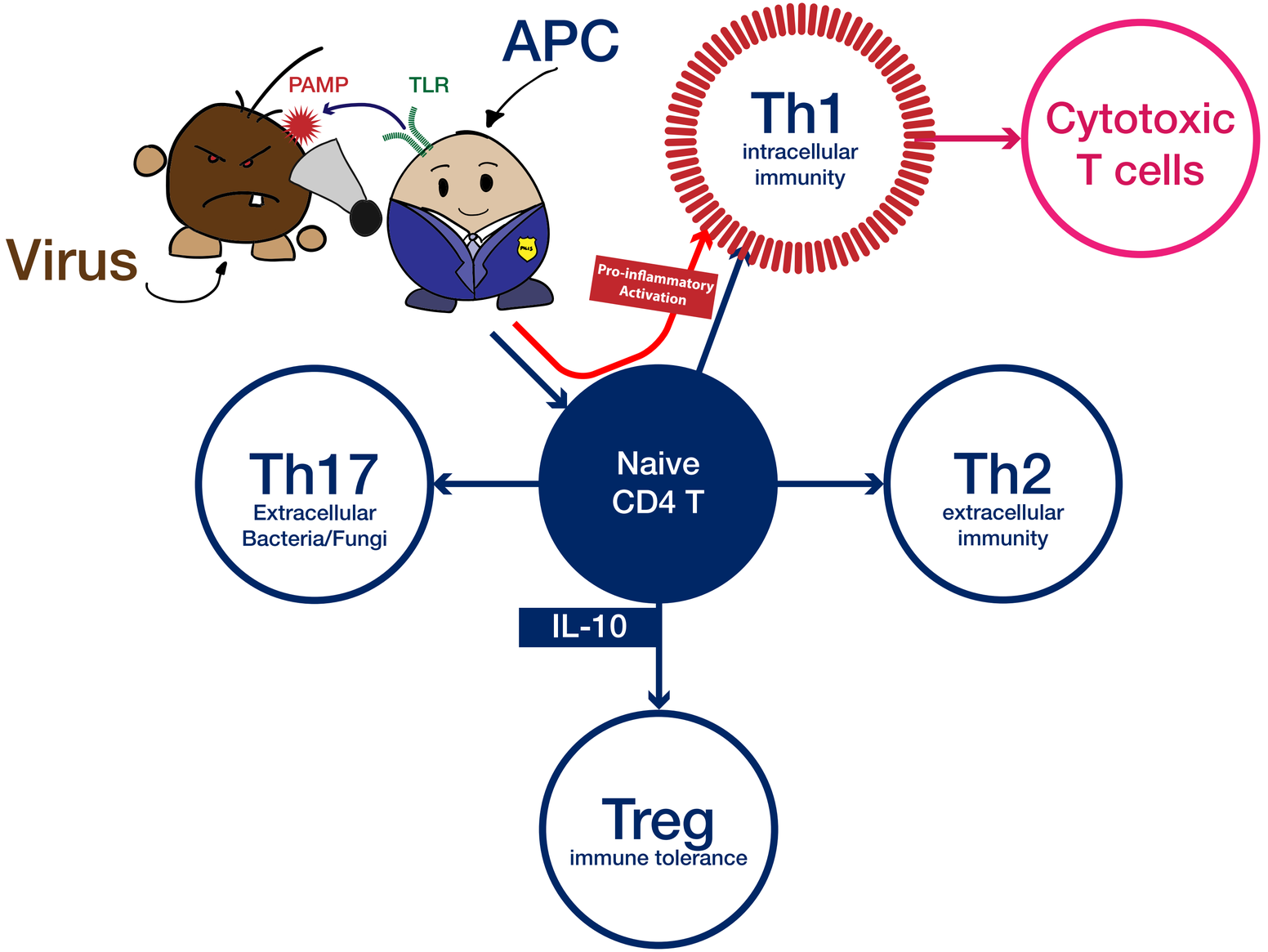
= uncontrolled inflammation

Storm of cytokines is excessive inflammation that results if we are not able to dampen the inflammatory communication between innate and adaptive immune system

The communication that was meant to activate our T-lymphocytes Communication that should be dampened by our anti-inflammatory systems: T reg & IL-10

Inflammation is not a single thing that simply goes up and down but it is an orchestration of interconnected processes with a choreography that includes the chemistry of activation and resolution

The inflammation drives our immune response but sometimes we see a rapid escalation in which inflammation gets excessive what will finally cause damage


APC
PAMP
TLR
Virus
Th1
intracellular
immunity
Cytotoxic
T cells
Pro-inflammatory
Activation
Th17
Extracellular
Bacteria/Fungi
Naive
CD4 T
Th2
extracellular
immunity
IL-10
Treg
immune tolerance

How do we improve T reg activity & self-tolerance?

- In autoimmunity
- In storm of cytokines

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↑

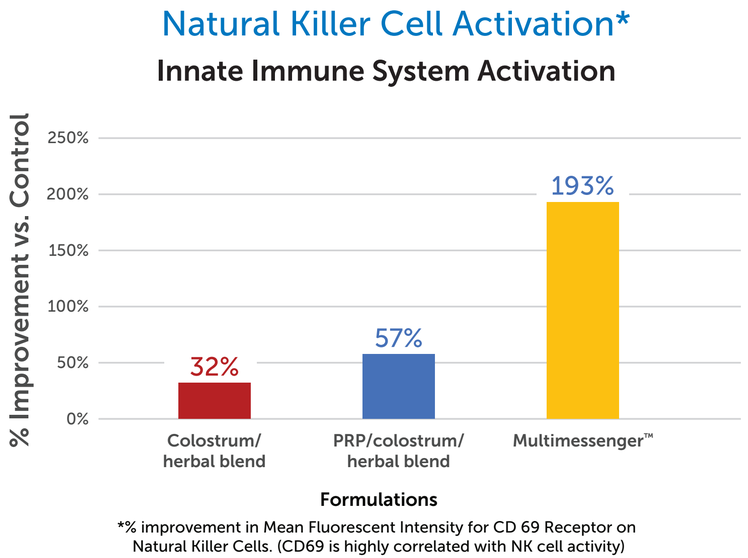
- **Optimizing DHEA** - individual

Transfer factors are like a cross between interleukins and antibodies

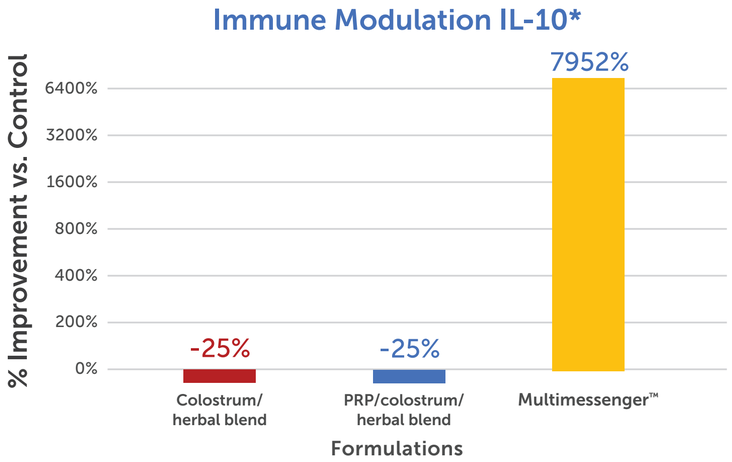
Carrying messages from immune cell to immune cell like interleukins

= General strengthening of Th1 & NK Cells

= rebuilding balance Th1/Th2/Th17 & downregulate autoimmunity



*% improvement in Mean Fluorescent Intensity for CD 69 Receptor on Natural Killer Cells. (CD69 is highly correlated with NK cell activity)



* % Improvement In Mean Fluorescent Intensity for IL-10 on Peripheral Blood Mononuclear Cell Cultures (PBMC)

Binding to antigens on infected cells like antibodies (= specific Transfer Factors)

Further advise in storm of cytokines

- **Pretreatment to lower inflammation and oxidative stress**
  - **EGCG reduces NF-kB activation**
    NF-kB is the key regulator of genes coding for inflammatory mediators
    Pre-treatment with EGCG decreased secretion of IL-1 beta, IL-6,
    TNF alpha & chemokines
    Resveratrol reduces and prevents inflammatory disorders next to an antiviral activity against human and animal viruses
  - **Oral Liposomal Glutathione**
    Published study shows Liposomal Glutathione levels oxidative stress and enhances Natural Killer Cell Activity with 400% after 2 weeks use
  - **Depending on individual presentation**
    - Phospholipid replacement therapy to restore mitochondria in post-viral fatigue
    - Rebuild normal coagulation pathway

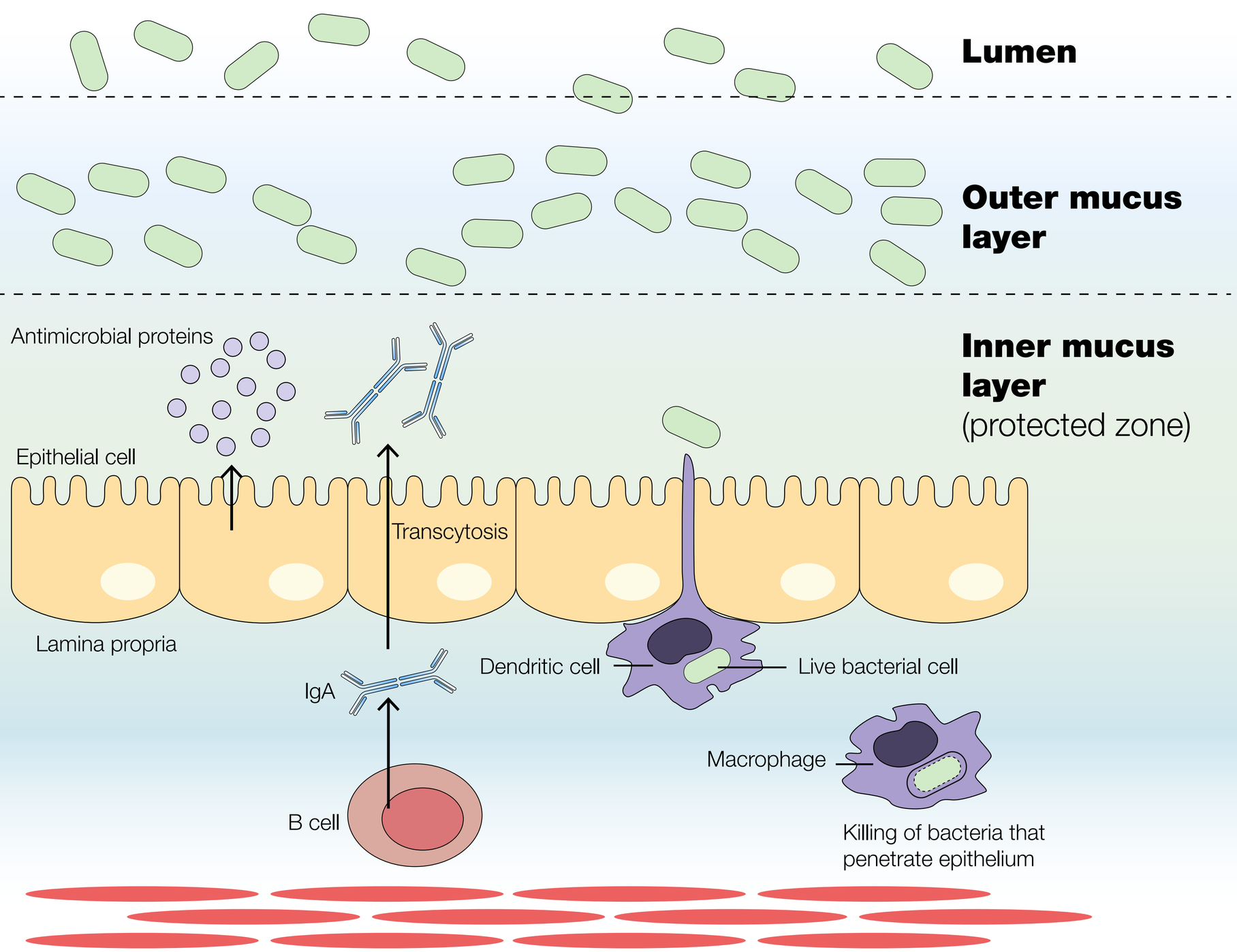
The evidence suggests that the pathology in severe viral infections is mediated by cytokine response not by viral load

The gastro-intestinal barrier is a multi-layered and integrated system, an essential part of our immune system.

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

- Analysis of the different layers of intestinal barrier: what are the primary components in each barrier?
- Show the mutual relation between microbiome and intestinal permeability
- Overview of the key regulating factors



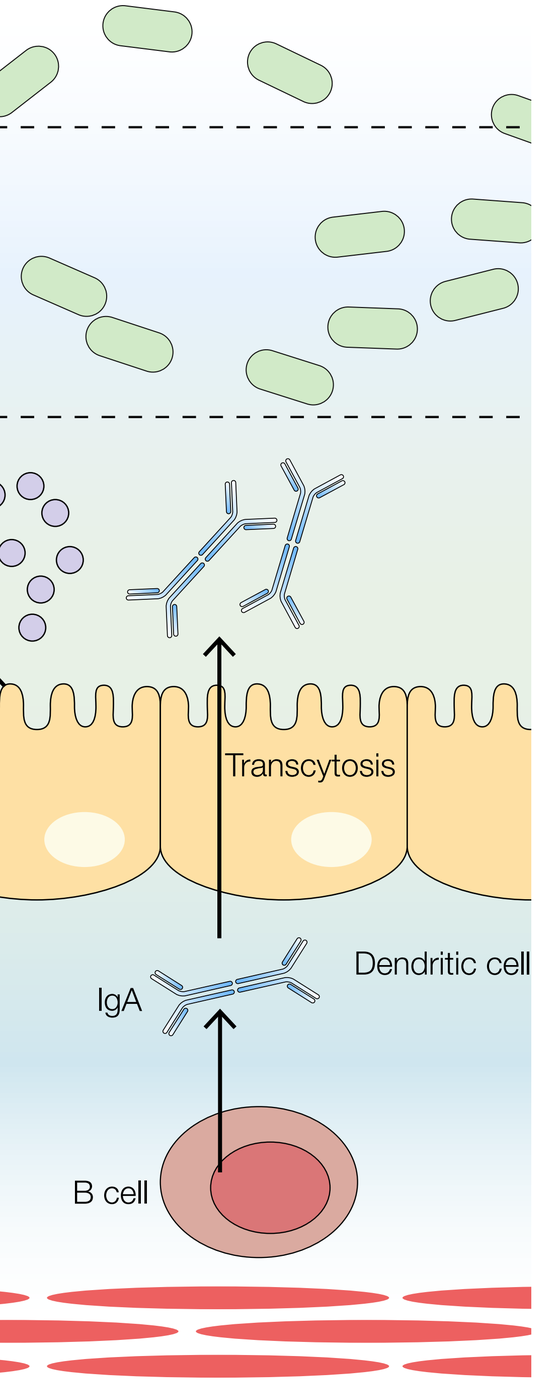
Microbial barrier
commensal bacteria

Chemical barrier
mucus layer

Physical barrier
the epithelium

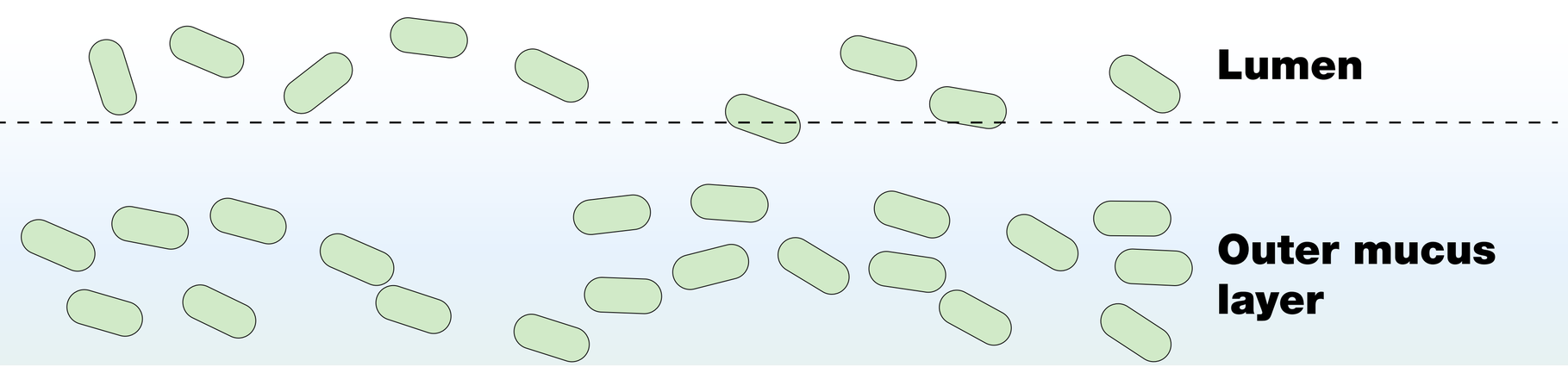
Immunological barrier
immune cells
of the lamina propria

Muscle layers
smoot muscle intestinall wall



The intestinal barriers form a huge multi-factorial, layered and highly integrated system with high maintenance:
40% of our daily energy volume is used to maintain our gut barriers

1. **Commensal bacteria and their metabolites**
2. **Functional biochemical barrier**
   immune molecules (s IgA, antimicrobial peptides) and inflammatory mediators (cytokines) in a mucus layer
3. **External physical barrier**
   Epithelial cell lining /tight junctions
   Underlying the epithelial lining, we have the lamina propria (contains the immune system of the gut), vascular endothelium



Microbial barrier
commensal bacteria

It’s a two way street
= Mutual regulation between microbes and barriers
= The commensal bacteria facilitates optimal status of the barrier components & in turn…

The barrier components provide surveillance, protection and selection of the commensal bacteria
+ eliminates harmful pathogens

Commensal bacteria?

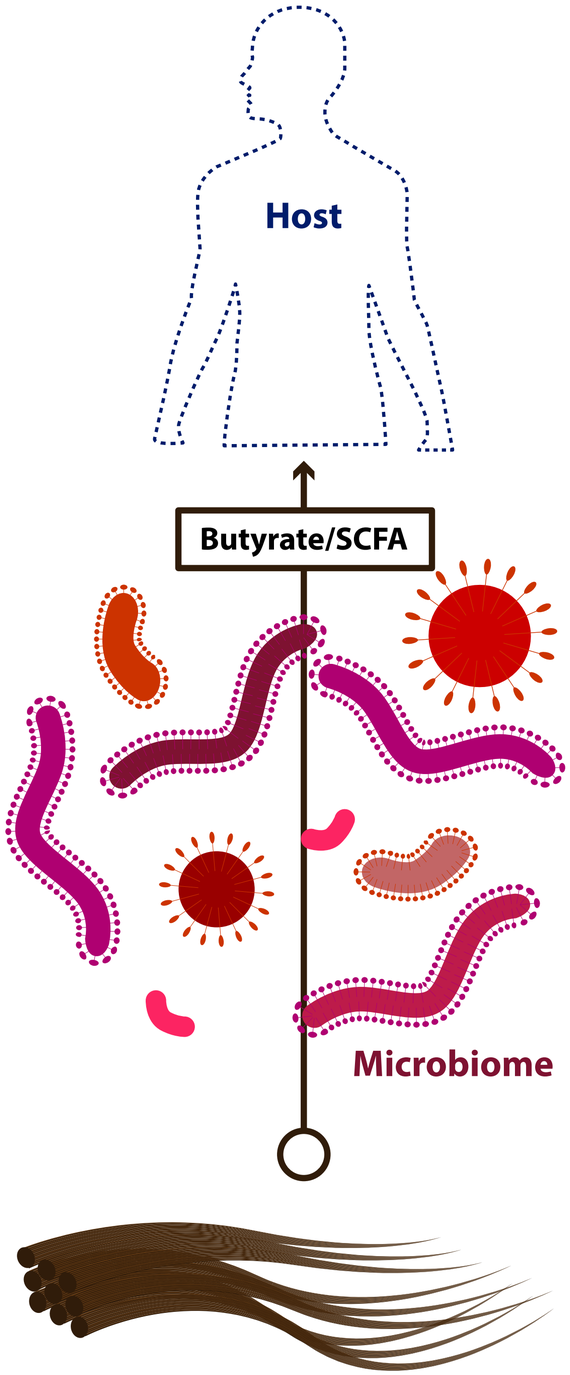
Example

- 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



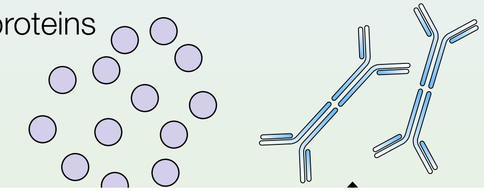
Major issue = Decrease in Butyrate production

butyrate is a major intestinal messenger

Loss of butyrate =
Microbial-host cross talk compromised
Compromised permeability

+ We get colonization of imbalanced Flora

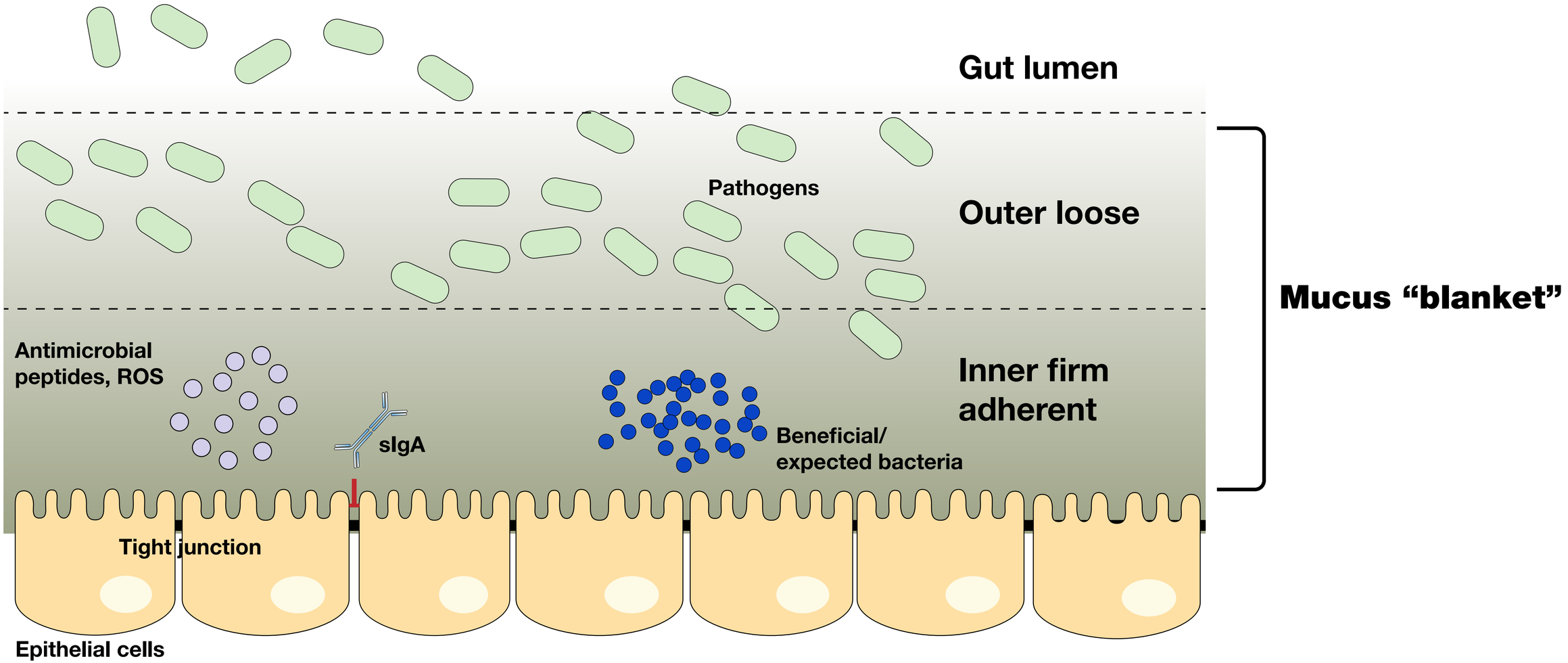
Antimicrobial proteins



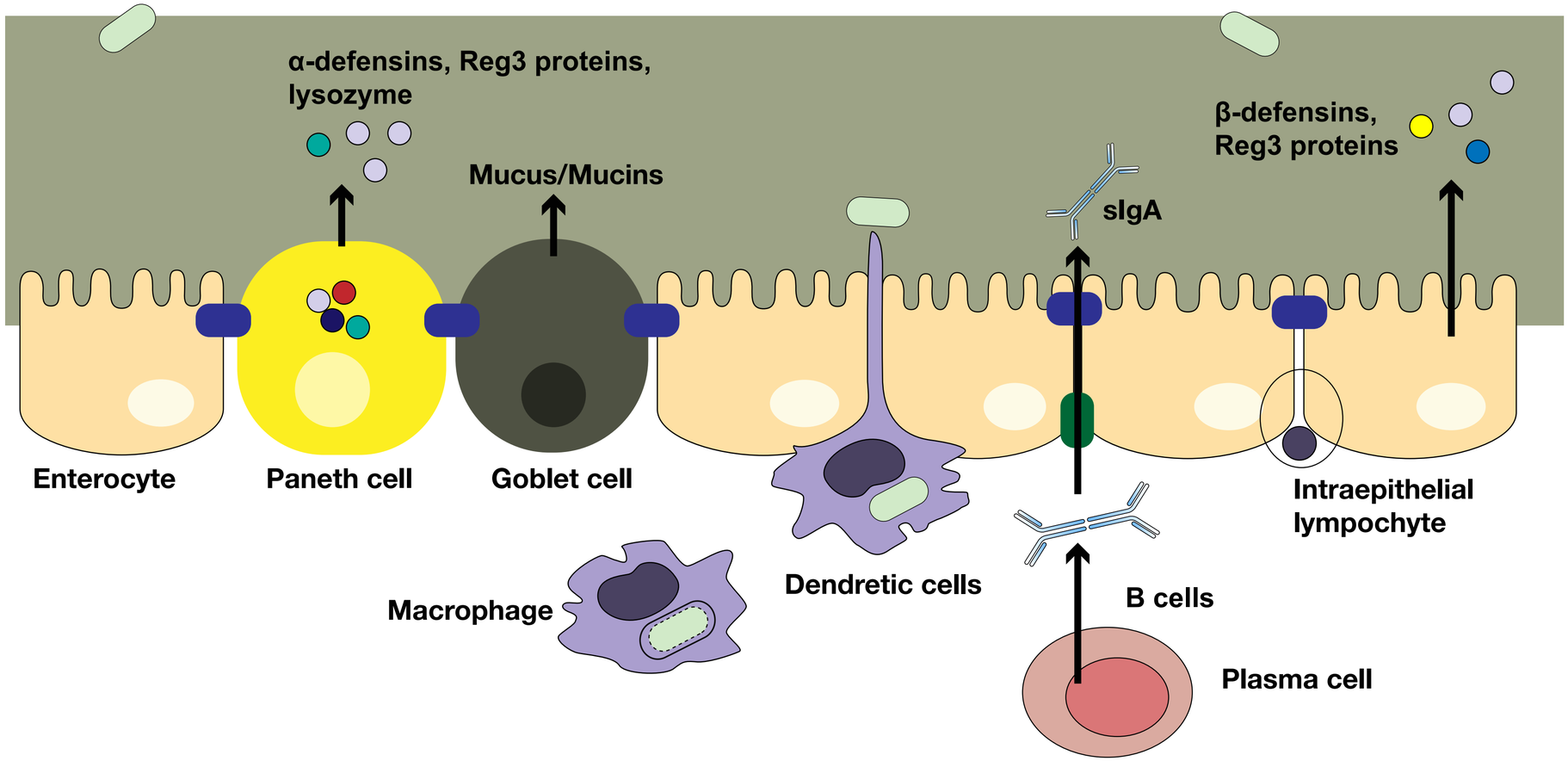
Inner mucus layer
(protected zone)

Chemical barrier
mucus layer

Mucus & Mucosal barriers -The Terrain



Entire system of microbial detection, recognition and eradication

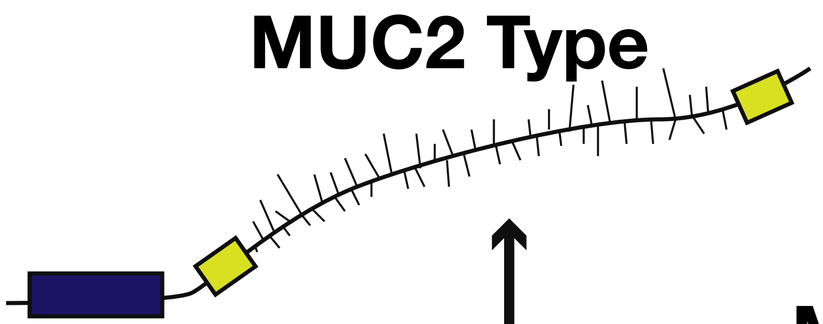


Mucins are highly glycosylated proteins, polymers form a gel-like network

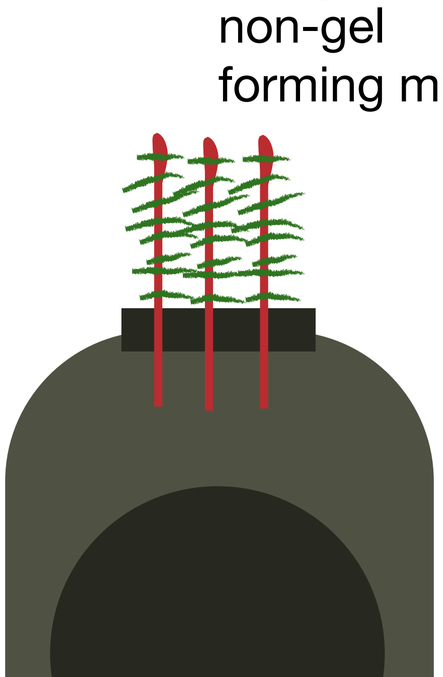
- Mucins are essential to maintain Gut Barrier function, mucins can be compared with biofilm
- Mucins are to the epithelial cells as the biofilm is to bacteria and yeast;
- Prevent direct bacterial binding to epithelial cells
- Mucins are regulated by commensal microbiota within the mucosa

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



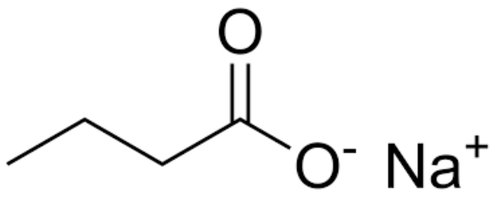
Gel
forming mucins



non-gel
forming mucins

MUC 1 type

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



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

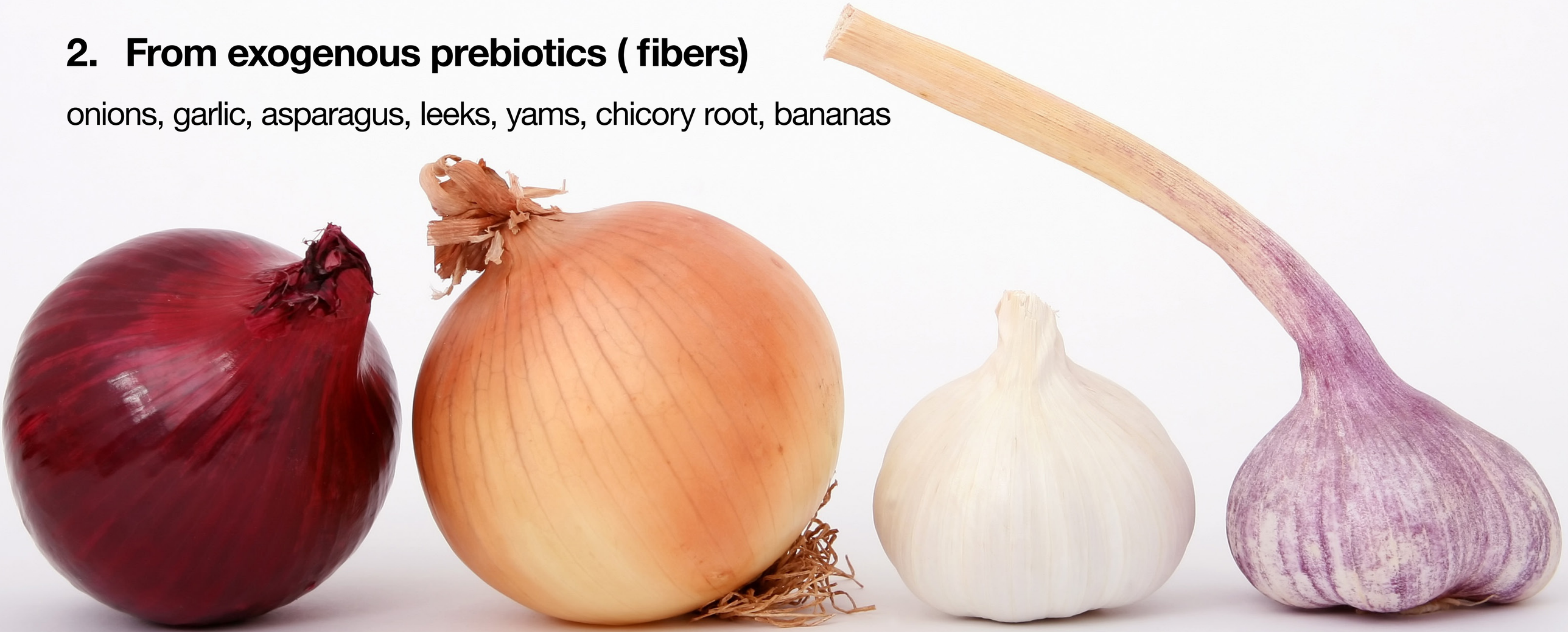
What bacteria produce butyrate?

- **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

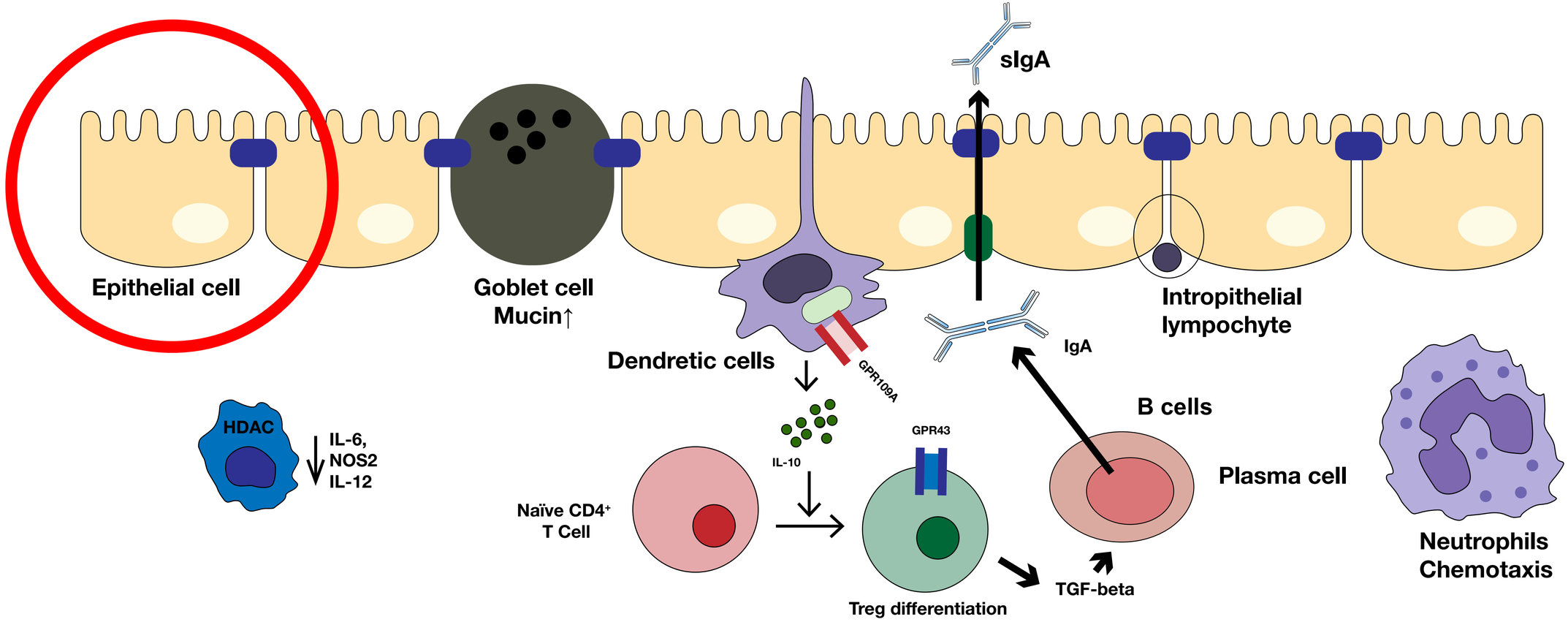


What is the role of butyrate and SCFA's?

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”

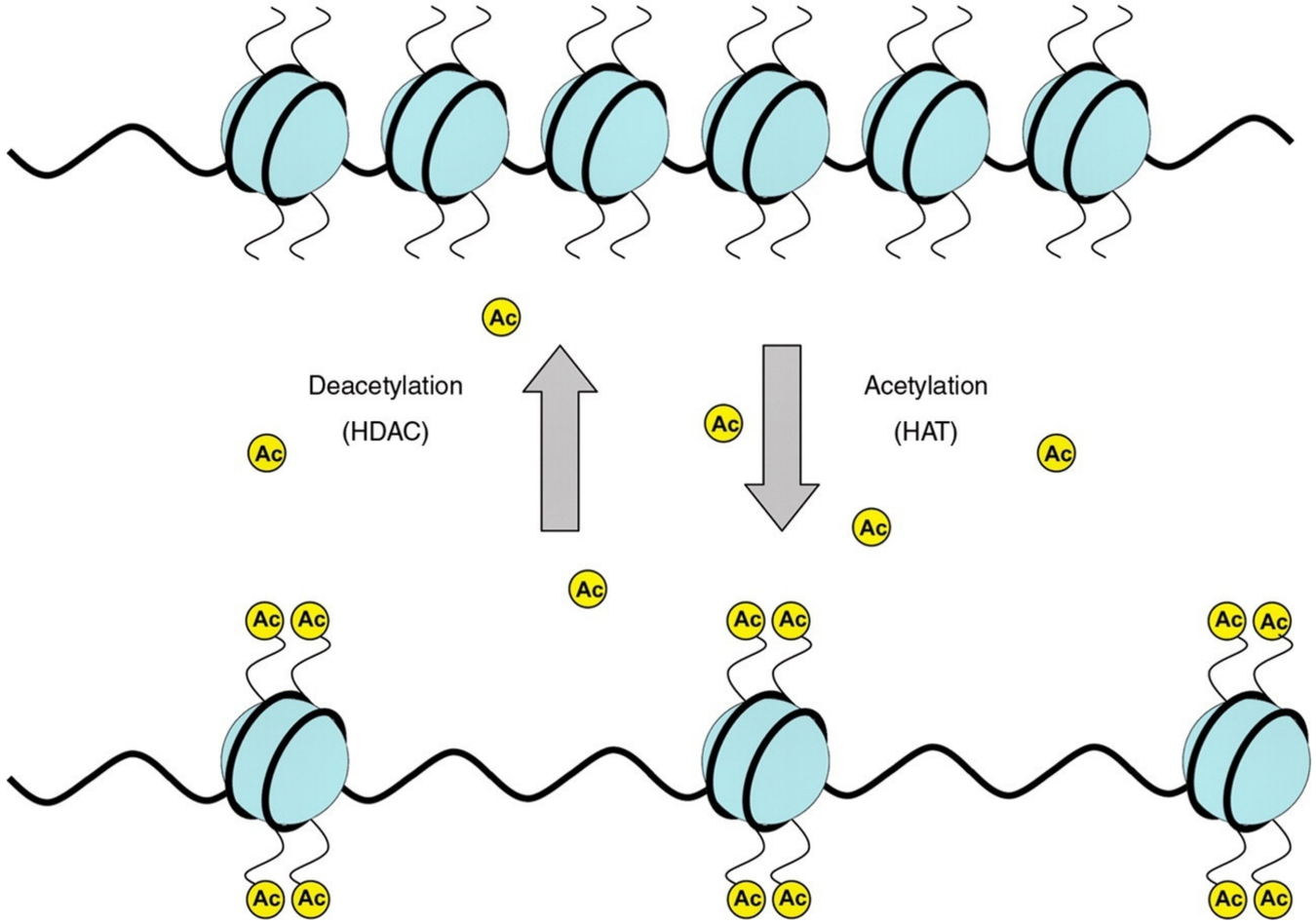
=Butyrate impacts epigenetics
= Butyrate modifies genetic material
→ impact on gene expression and transcription

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.

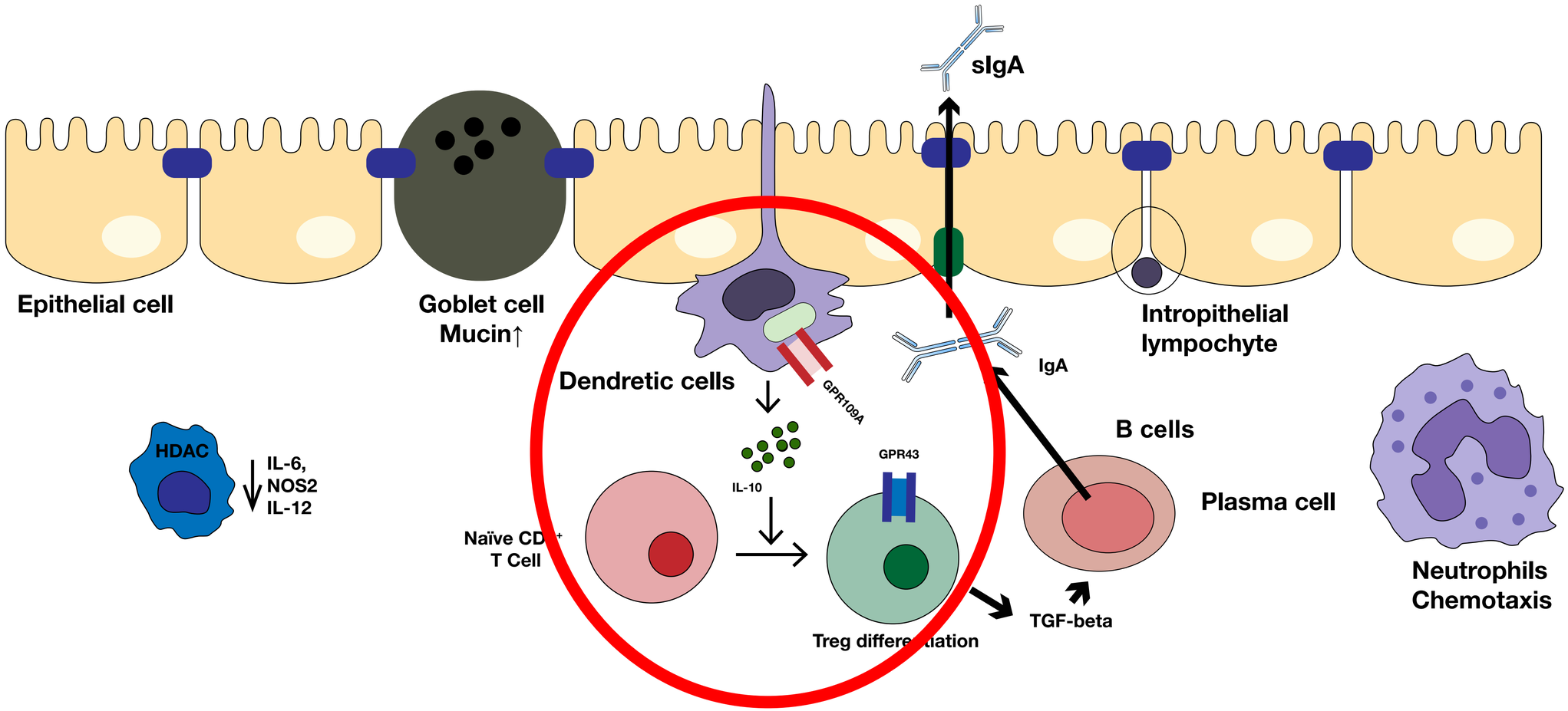
Immune modulation / anti inflammation on local level:
Butyrate inhibits HDAC (histone deacetylase) – this modification is changing the gene expression



Gene expression is modified in Dendritic Cells

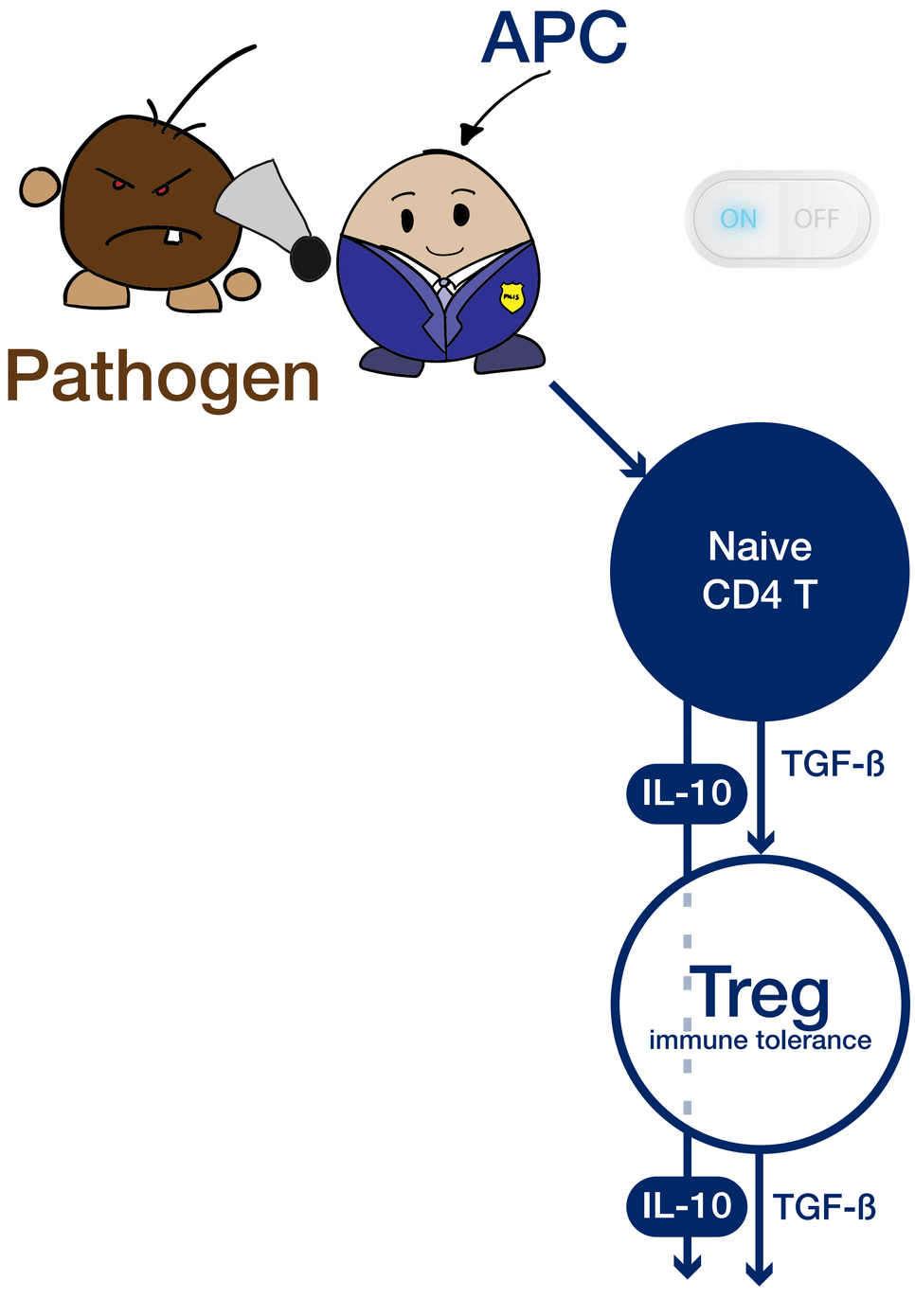
IL-6 is suppressed = more IL-10

More differentiation to T regs



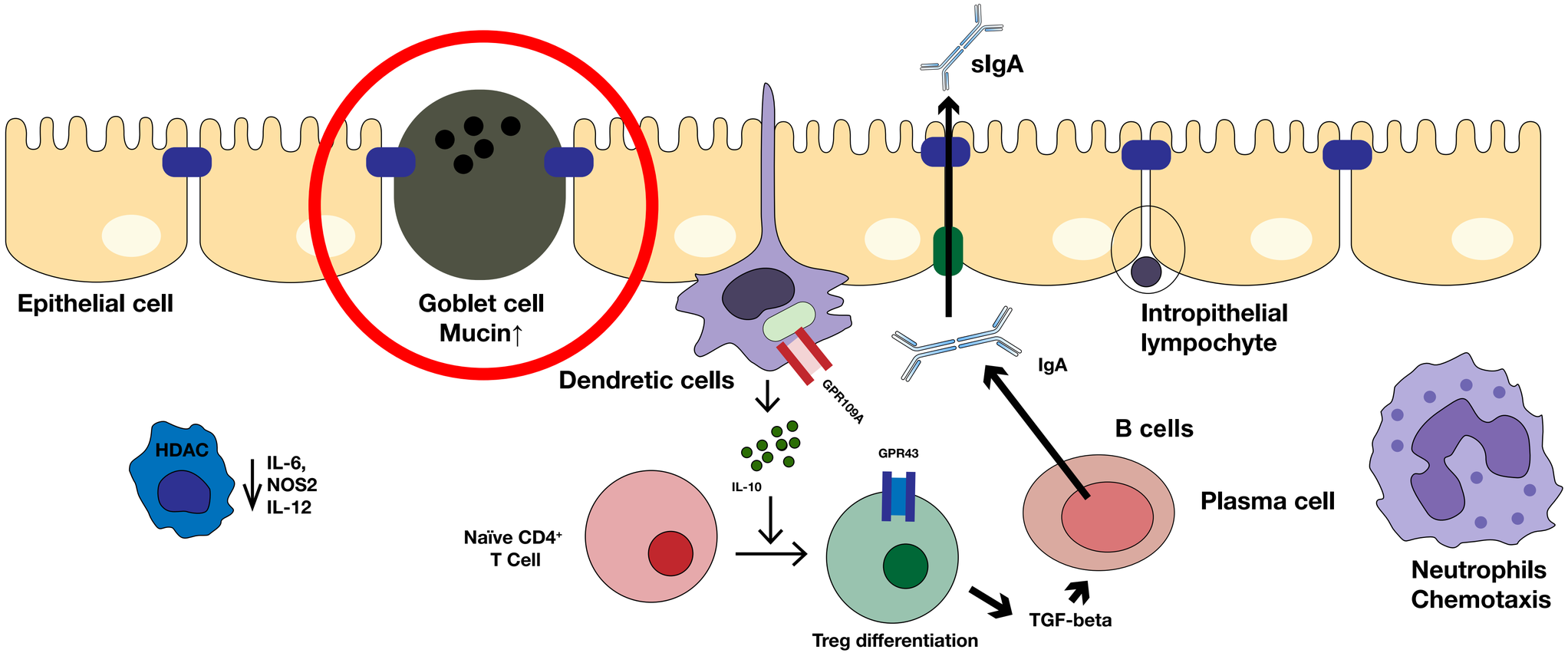
T reg & IL-10 = self tolerance

- ↓ Risk for autoimmune response
- ↓ Risk for excessive inflammation



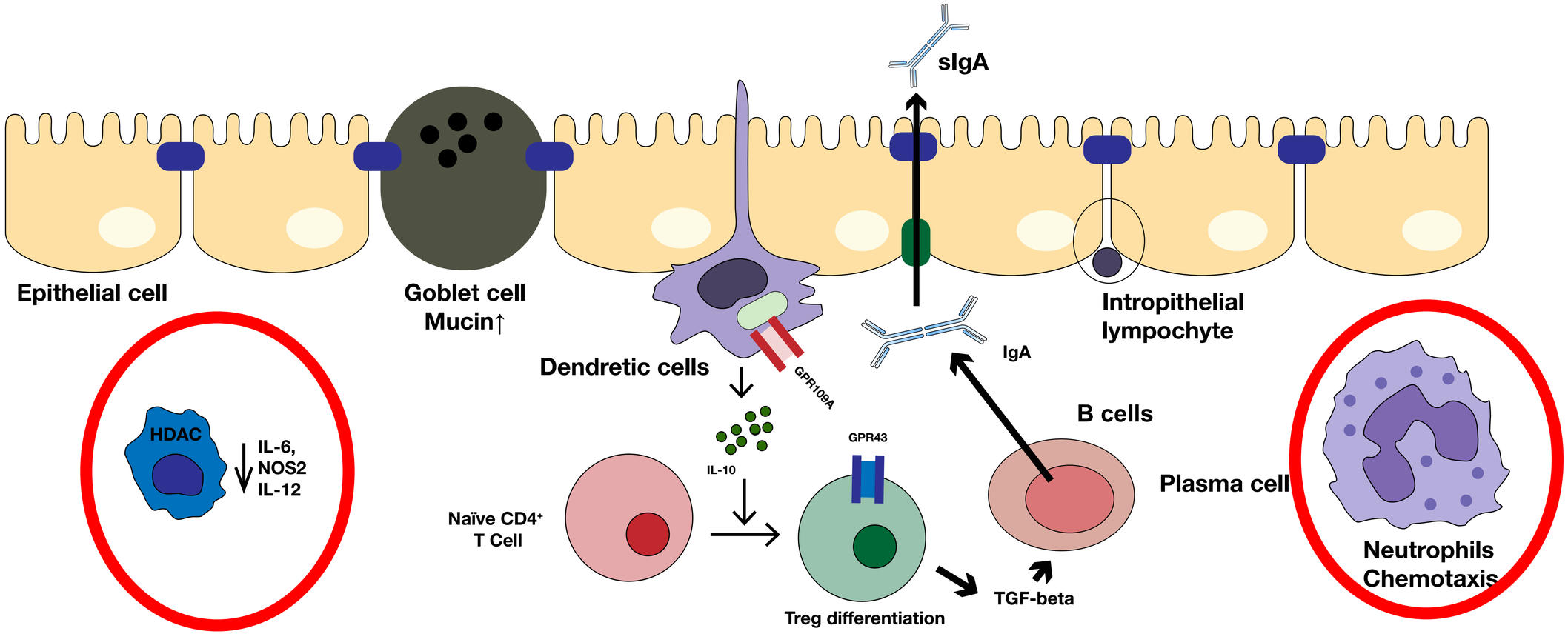
Differentiation of Goblet Cells and mucus formation

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

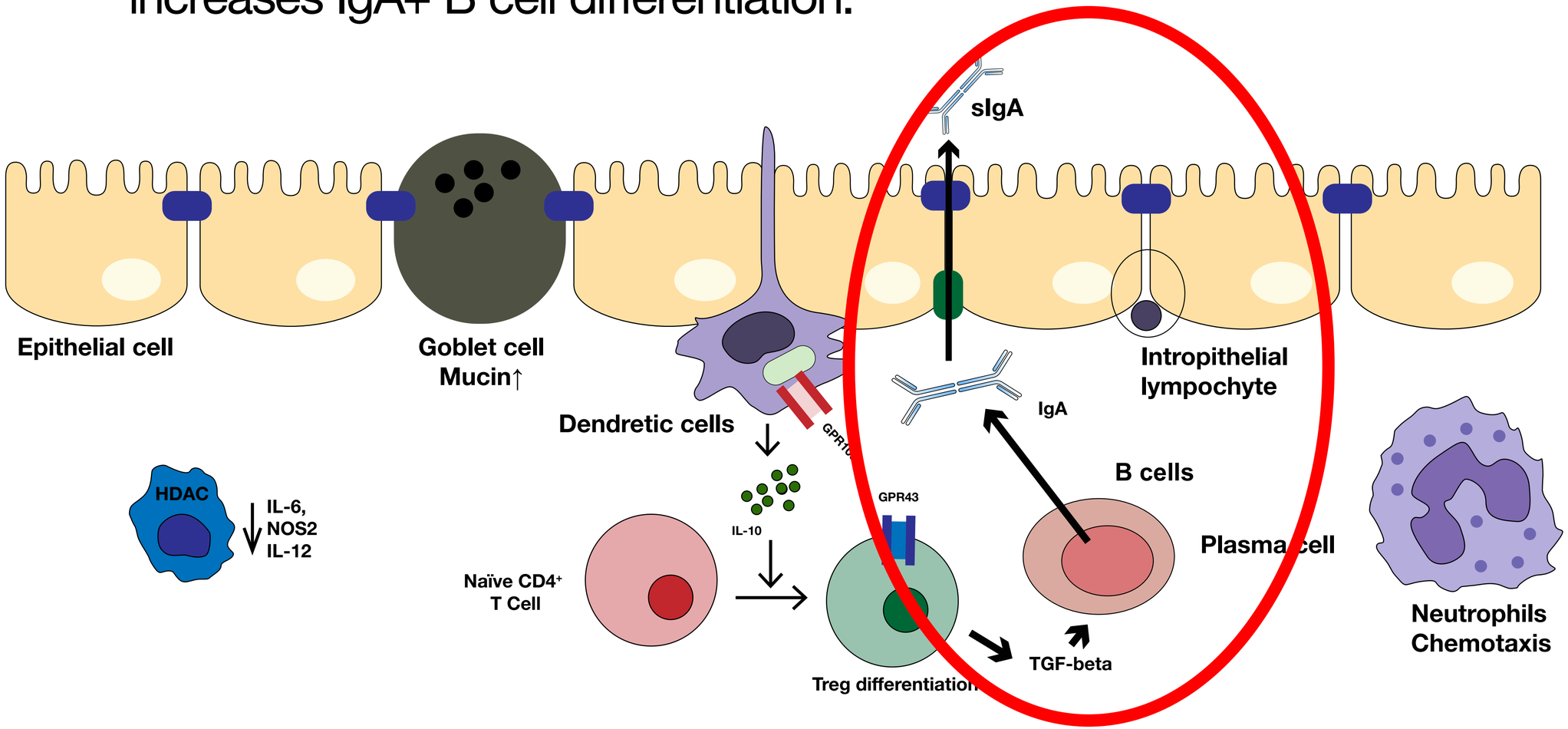
Butyrate affects neutrophil chemotaxis anti inflammation on local level



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

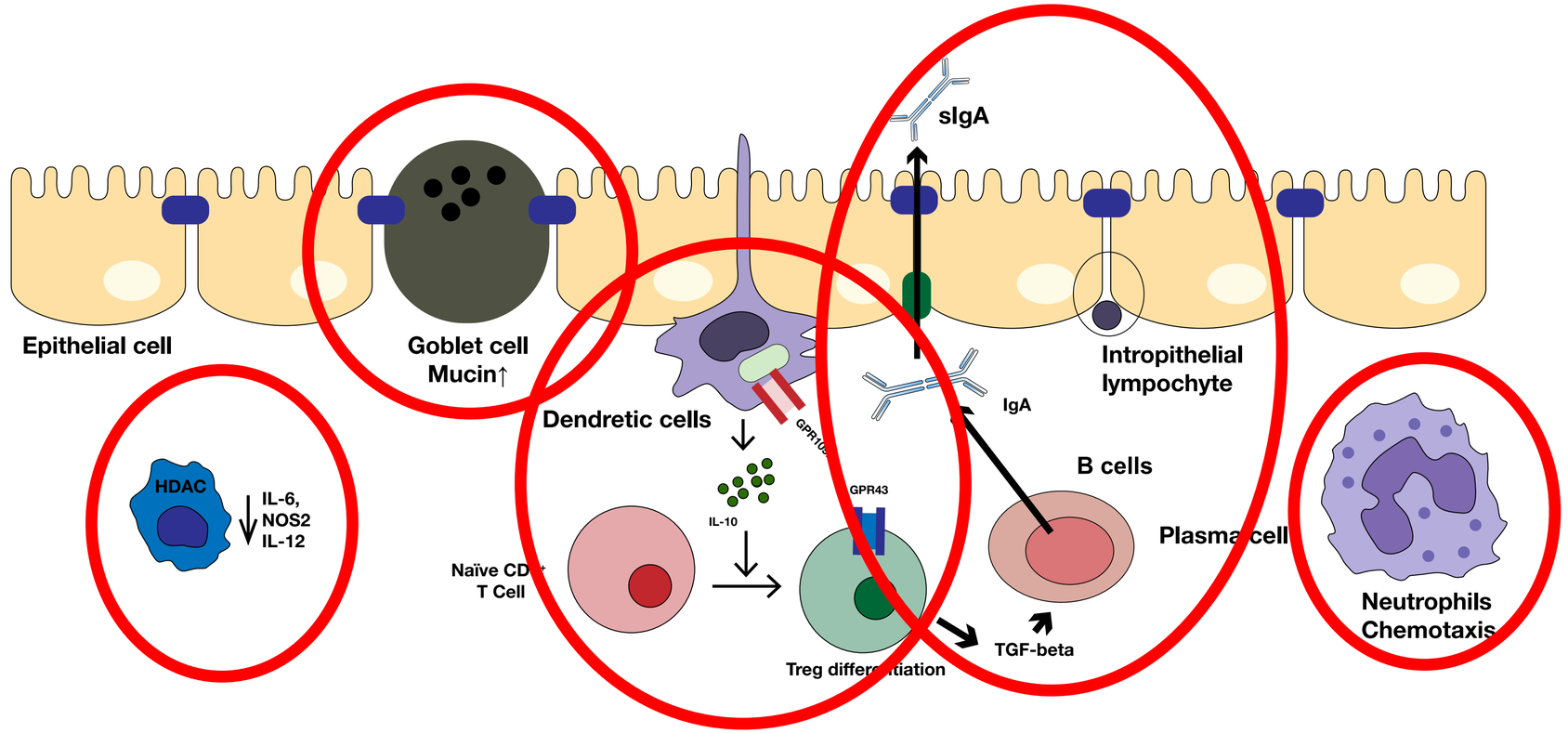
Impact on dendritic cells, more IL-10 & T regs

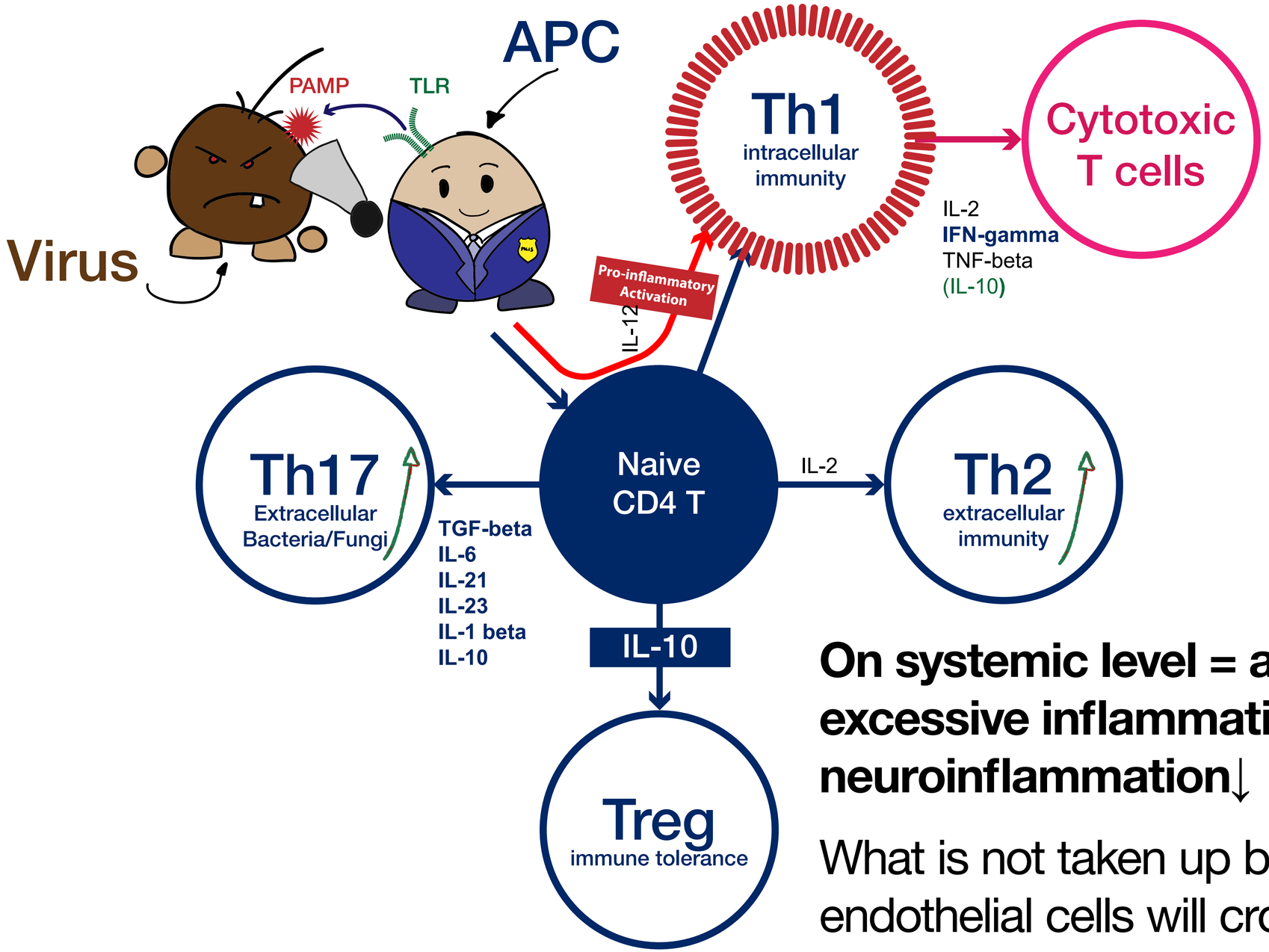
Goblet Cells release more mucins

Macrophages more tolerant towards commensal bacteria

Neutrophil chemotaxis

B cells synthesize more s IgA's





On systemic level = autoimmunity↓, excessive inflammation↓ & neuroinflammation↓

What is not taken up by enterocytes and endothelial cells will cross into systemic circulation and even the blood brain barrier

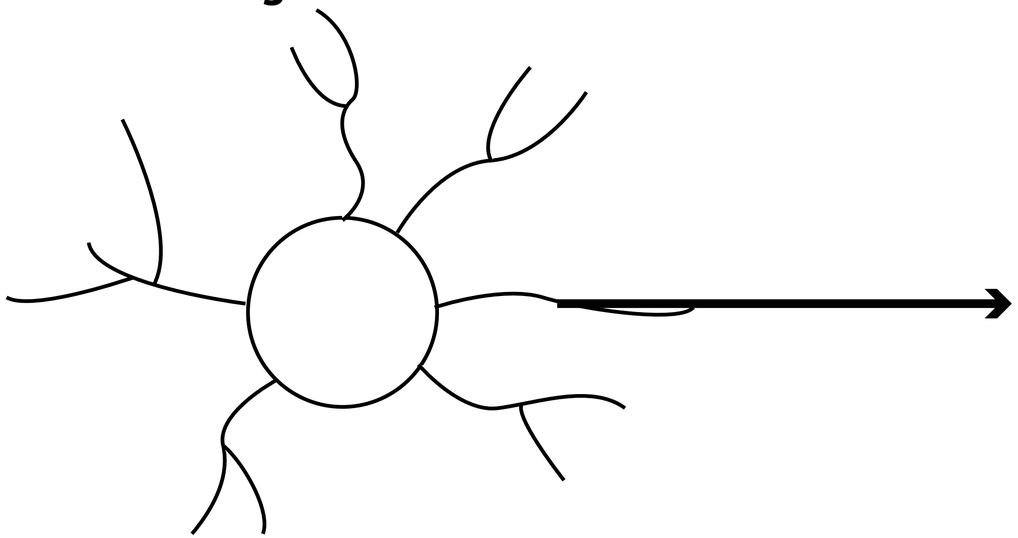
IL-10 favors the differentiation of naïve CD4 cells into T regs

Tregs dampen excessive manifestation of immunity / autoimmunity

Mice deficient in IL-10 showed manifestations of local autoimmune conditions and systemic autoimmunity

Butyrate levels the release of inflammatory cytokines released by microglia
= modulation of neuroinflammation

Microglia in surveillance



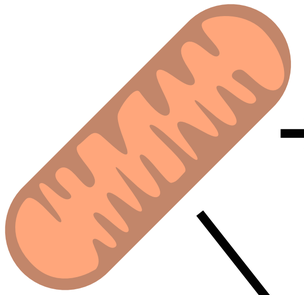
SCFA

Pro-inflammatory mediators
Il-1β, TNFα, IL-6 IFN-γ, δ-LoxCOX-2

if release of inflammatory mediators is chronic & sustained

Neuro toxic factors
$ONOO^-$
ROS
NO
Glutamate

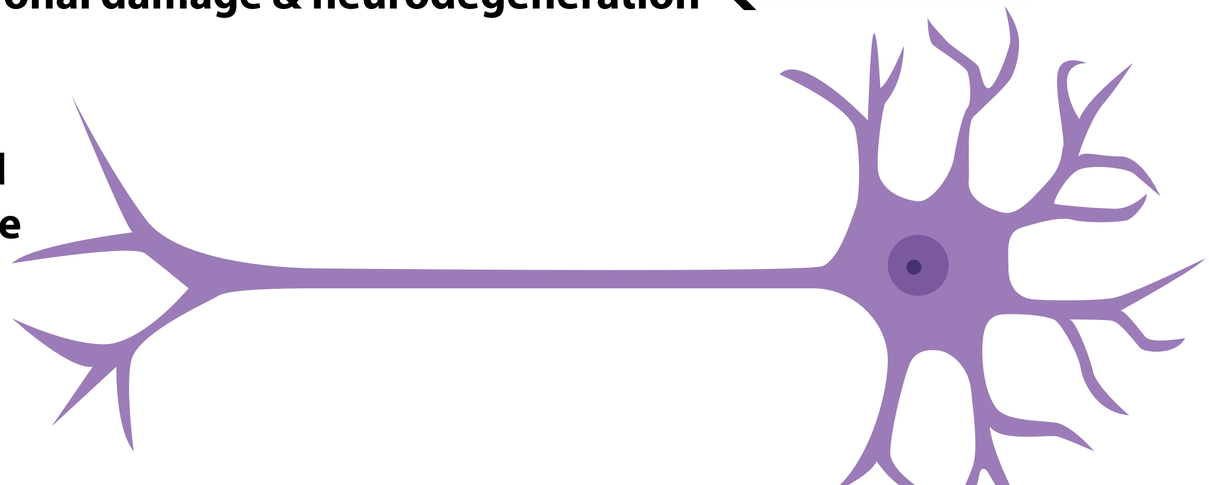
Neuronal damage & neurodegeneration

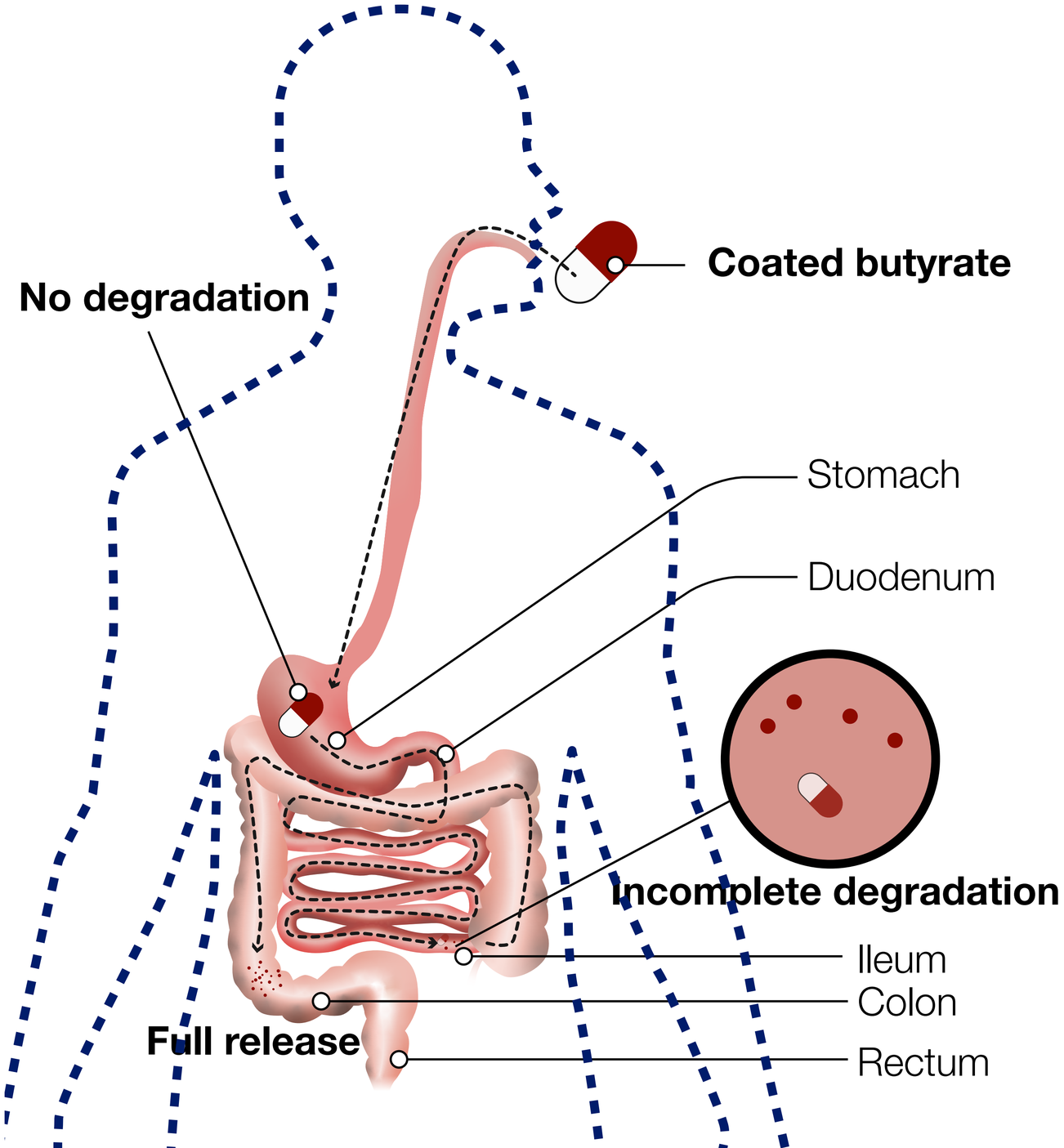


OH-

O_2^-

ATP synthesis blocked
Mitochondrial damage
Apoptosis induced





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 κB activation and tumor necrosis factor κ 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. Helmrath. “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.

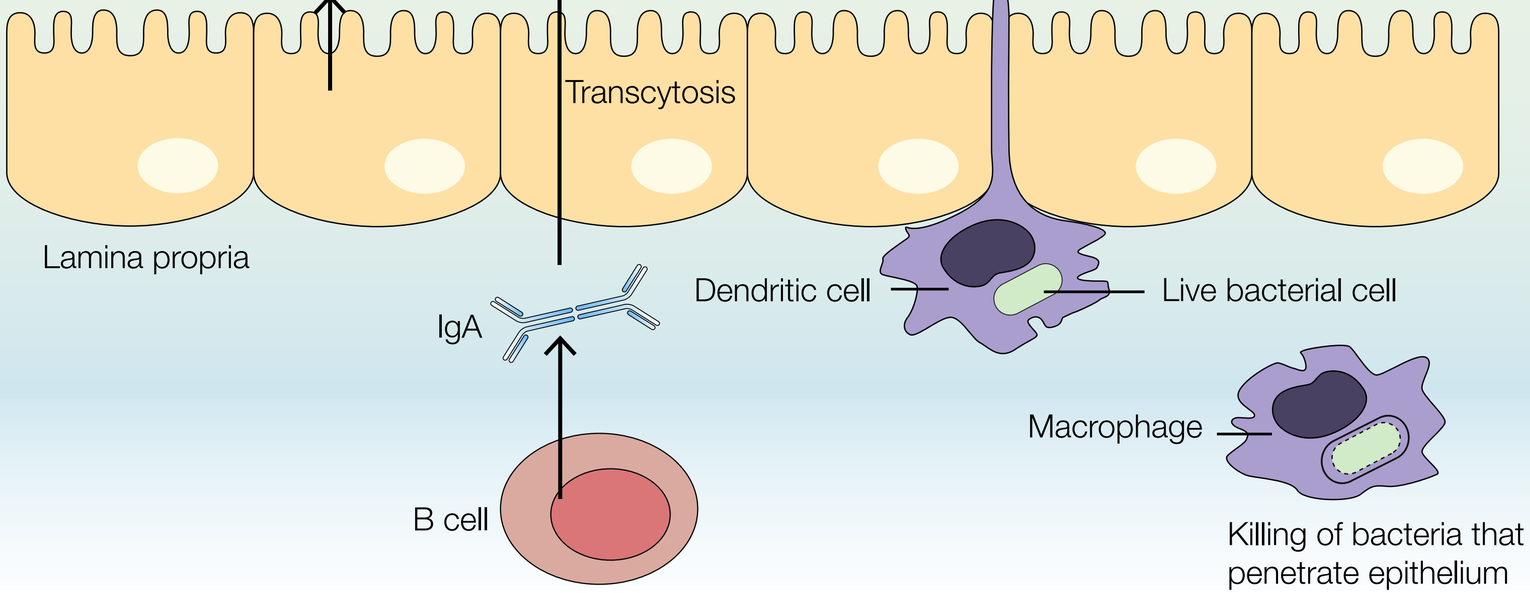
Wilson, Mark S., et al. “Colitis and intestinal inflammation in IL10–/– mice results from IL-13Rκ2–mediated attenuation of IL-13 activity.” Gastroenterology 140.1 (2011): 254-264.

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 shortκchain 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.



Physical barrier
the epithelium

Immunological barrier
immune cells
of the lamina propria

B Cells release s IgA's

S IgA = the brick in the mucus barrier
What is the role of s IgA's?

- Immune exclusion of microbes and toxins
- Neutralizes LPS
- Activates eosinophils to attack parasites
- Constant surveillance
- transmitted from mother to child through breastmilk
- Levels of fecal s IgA are low in Chronic Candidiasis

interventions for low s IgA

- glutamine targeted release
- Butyrate
- Probiotics
  Soil based probiotics (resistant to stomach acid, resistant to antibiotics, not milk derived, high colonization grade), Lactobacillus rhamnosus, Bifidobacterium lactis, Saccharomyces boulardii
- Omega 3
- Vitamin D3

Stress is decreasing sIgA

When we talk about leaky gut, we are down to the physical barrier, the tight junctions…

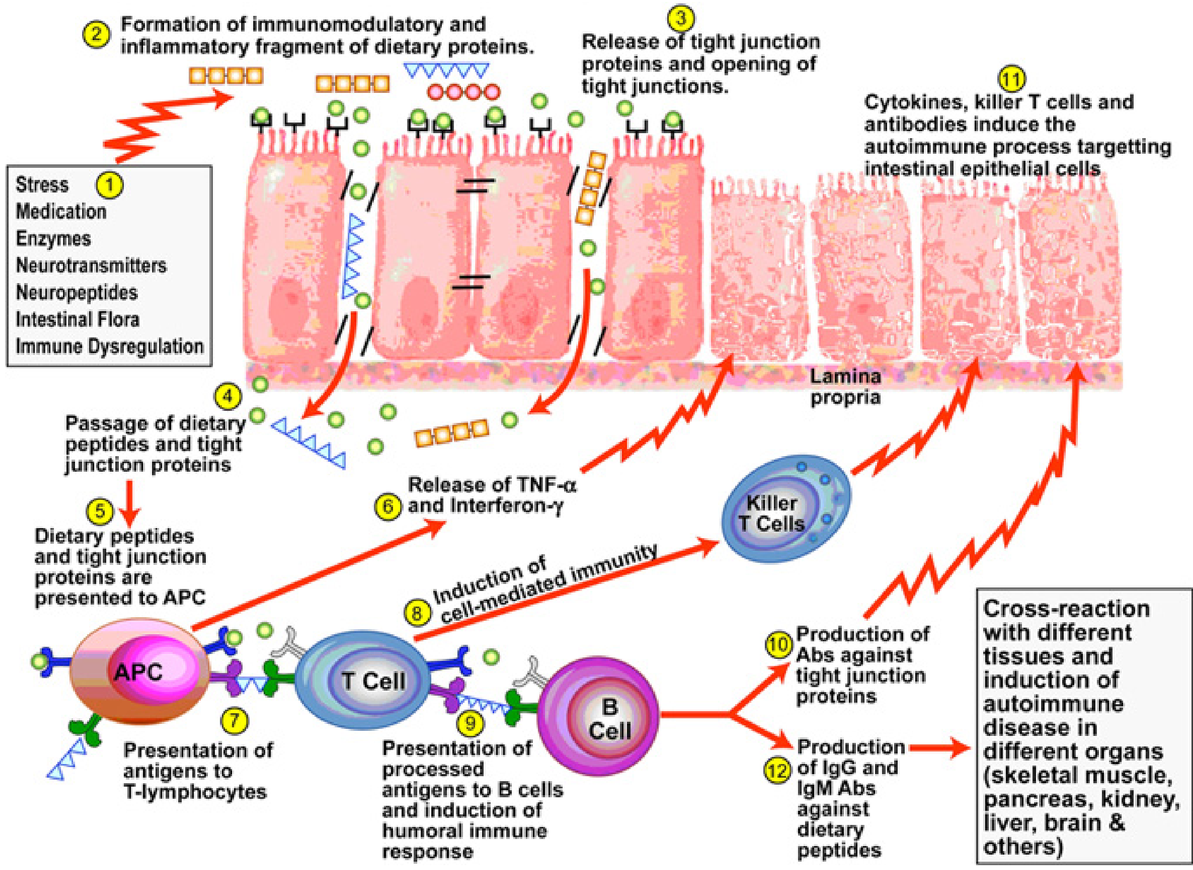
The epithelial barrier is a critical border preventing luminal material from entering the tissues.

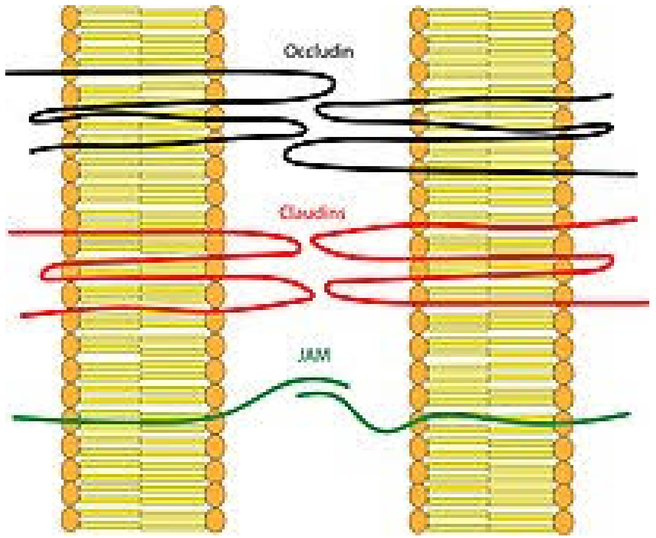
Essential components of this barrier are the **tight junctions, the seals between the epithelial cells.**

Tight junctions

- Restrict most microbes from penetrating
- Open to allow a robust response of the underlying immunity

A sustained opening of the tight junctions occurs in chronic disease:
autoimmunity, Celiac disease, cancer, Crohn's, MS, metabolic syndrome



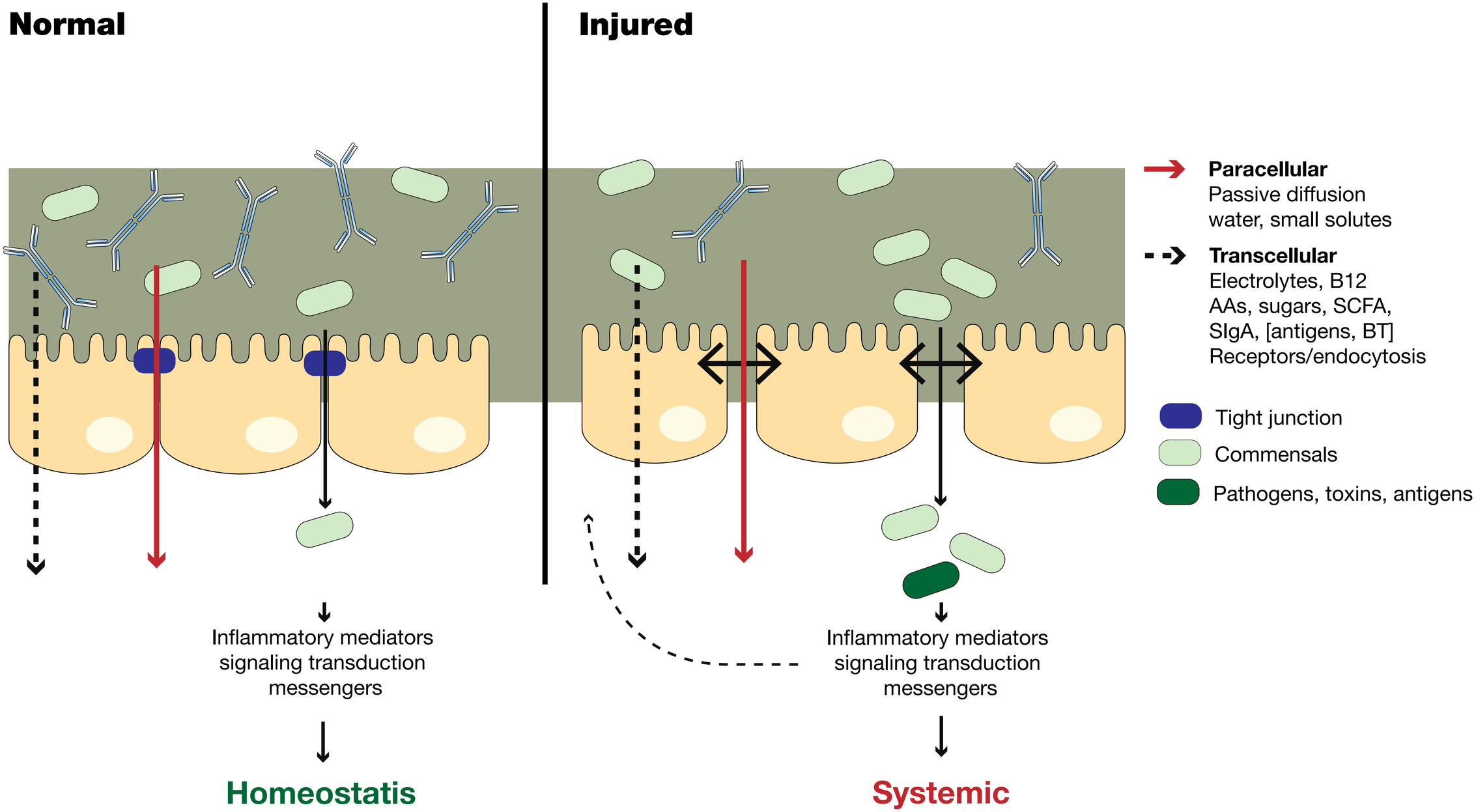


- Tight junctions are composed of a branching network of sealing strands

  Each strand is formed from a row of transmembrane proteins embedded in both plasma membranes

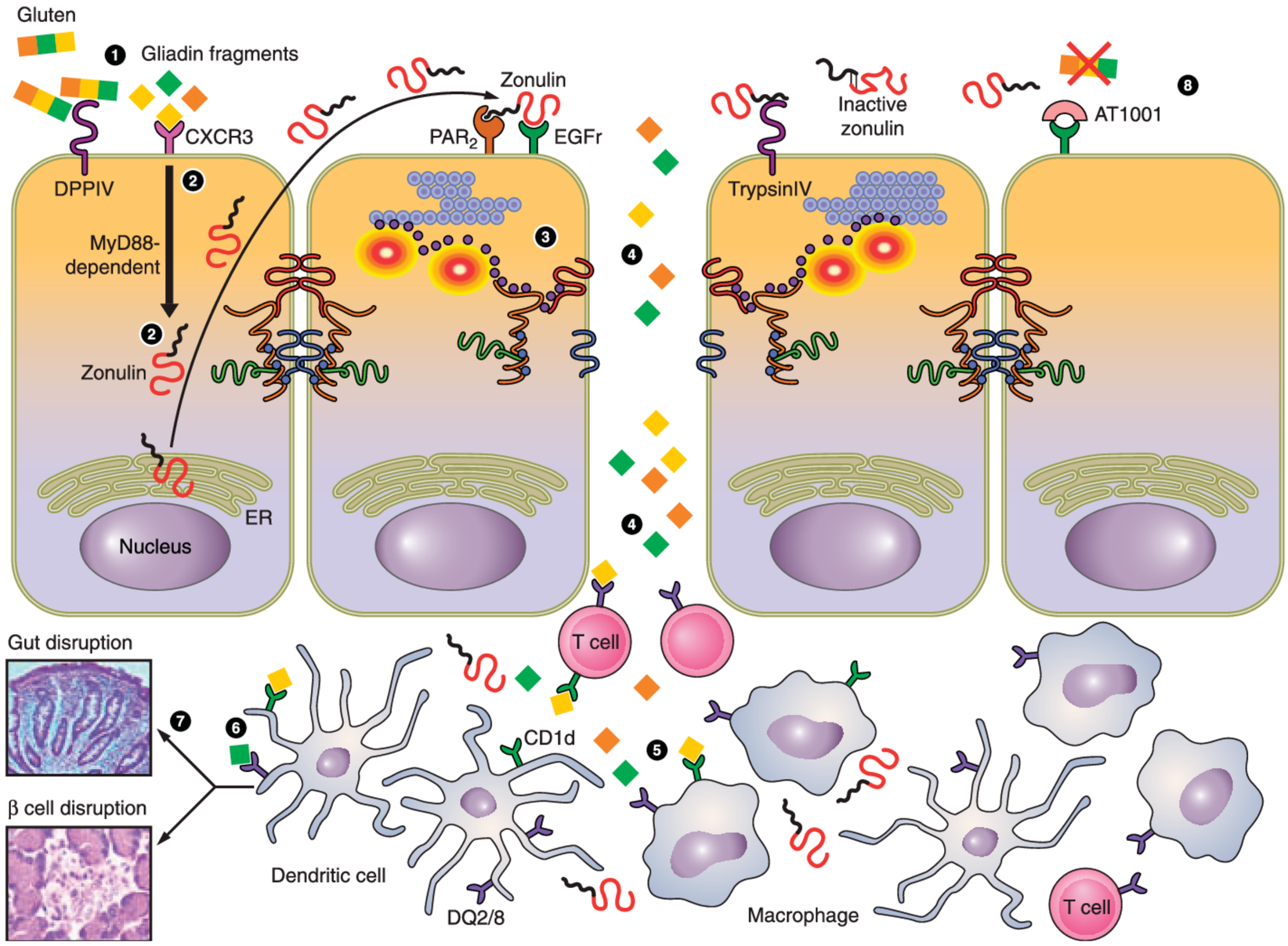
  Occludin and Adhesin are the main membrane proteins

- **Tight junctions regulate paracellular influx**


Normal
Injured
Paracellular
Passive diffusion
water, small solutes
Transcellular
Electrolytes, B12
AAs, sugars, SCFA,
SIgA, [antigens, BT]
Receptors/endocytosis
Tight junction
Commensals
Pathogens, toxins, antigens
Inflammatory mediators
signaling transduction
messengers
Inflammatory mediators
signaling transduction
messengers
Homeostatis
Systemic

- **Zonulin is the only physiological reversible mediator controlling the activity of the tight junctions**
  - Zonuline release is a diagnostical lab marker for leaky gut
  - Gluten/Gliadin is increasing zonulin release

Gliadin-induced Zonulin Release



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.

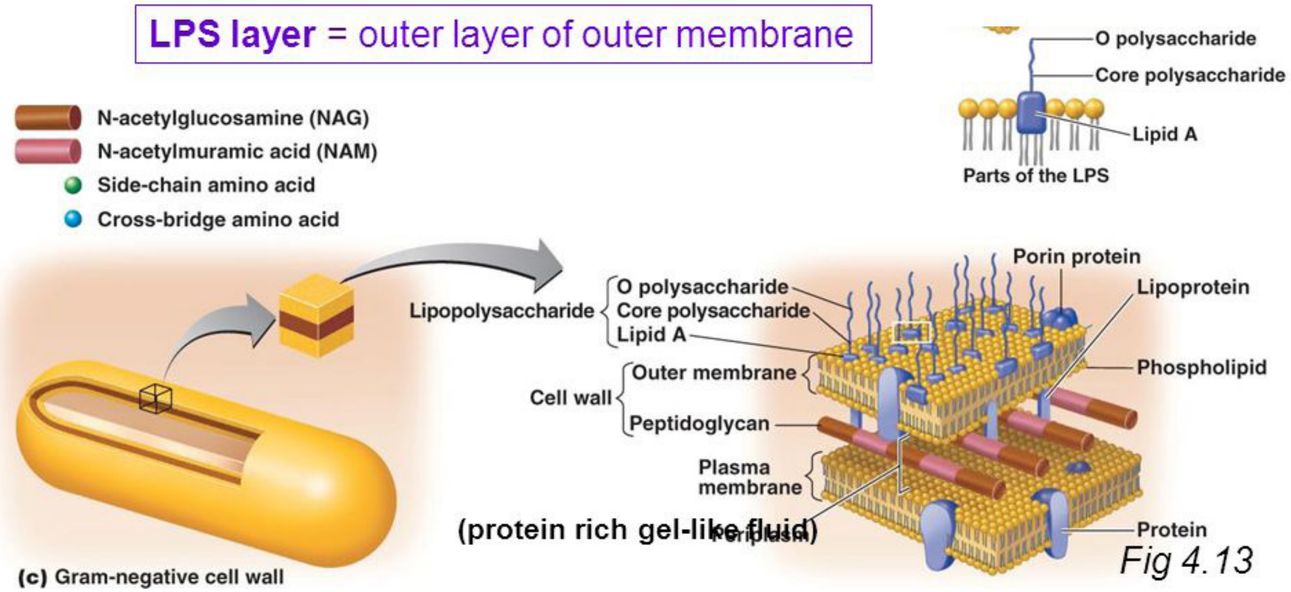
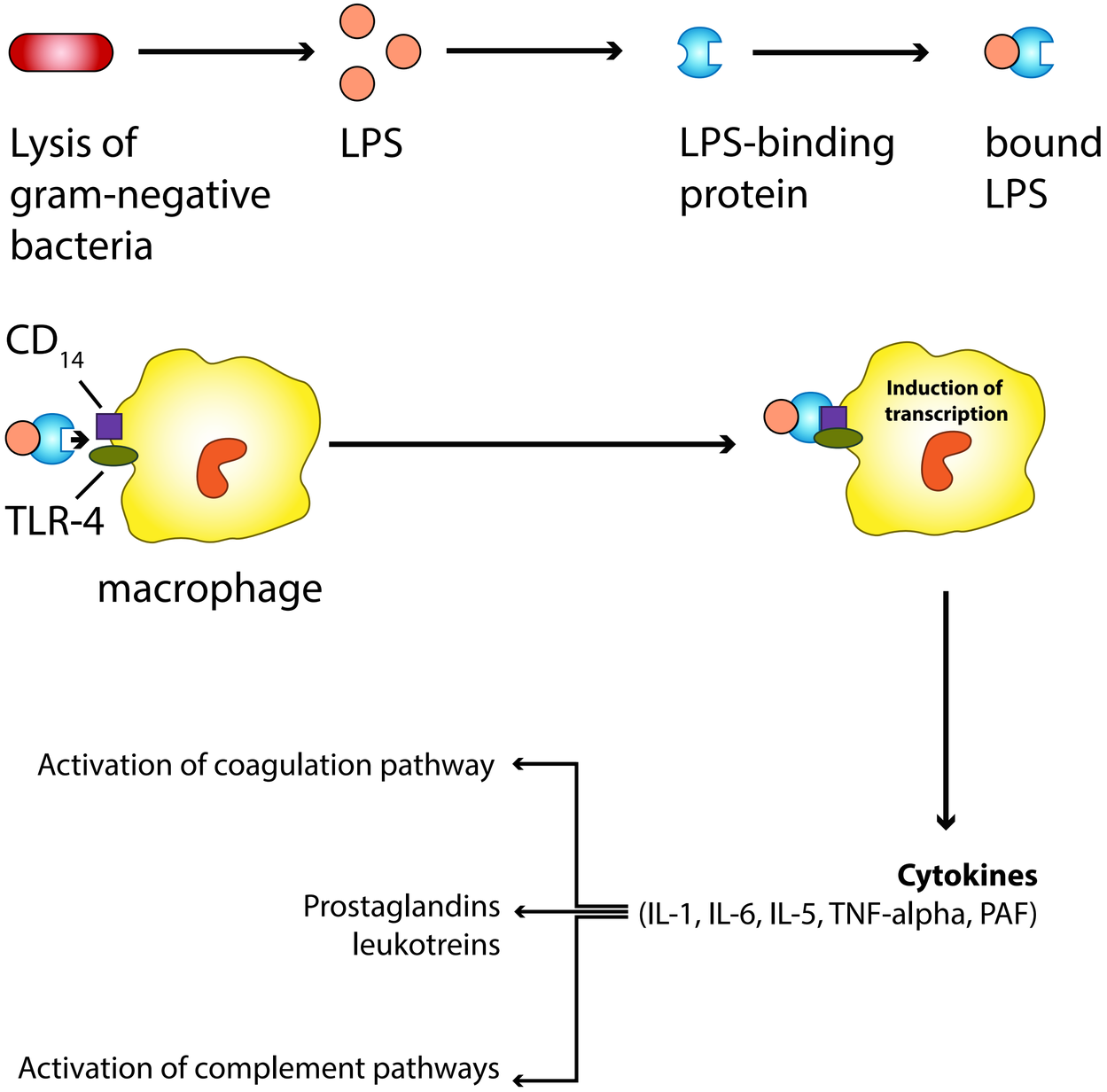


Fig 4.13


Lysis of gram-negative bacteria
LPS
LPS-binding protein
bound LPS
CD14
TLR-4
macrophage
Induction of transcription
Cytokines
(IL-1, IL-6, IL-5, TNF-alpha, PAF)
Activation of coagulation pathway
Prostaglandins leukotreins
Activation of complement pathways

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

A gluten- free diet benefits all patients with elevated serum-zonulin levels: IBS, Celiac and non-celiac Gluten sensitive

Barbaro, M. R., et al. **“Zonulin serum levels are increased in non-celiac gluten sensitivity and irritable bowel syndrome with diarrhea.”** (2015): S56-S56.

Increased Zonulin (increased permeability)

Other triggers than Gliadin

- IL-6
- Corticosteroids
- Stress
- Dietary protein fragments
- Fructose
- Microbes and toxins

Fasano, Alessio. “Intestinal permeability and its regulation by zonulin: diagnostic and therapeutic implications.” Clinical Gastroenterology and Hepatology 10.10 (2012): 1096-1100.

Corelation between increased intestinal permeability & autoimmunity

- **Persistent activation of zonulin pathway predisposes individuals to autoimmunity:**
  onset of diabetes type 1 was preceded by intestinal hyperpermeability
- **Blocking of zonulin binding to enterocytes with a synthetic peptide**
  - Decreased the incidence of diabetes type 1
  - Ameliorates symptoms in autoimmune pathology

Vanuytsel, Tim, Séverine Vermeire, and Isabelle Cleynen. “The role of Haptoglobin and its related protein, Zonulin, in inflammatory bowel disease.” Tissue barriers 1.5 (2013): e27321.

Kuitunen, Mikael, et al. “Intestinal permeability to mannitol and lactulose in children with type 1 diabetes with the HLA-DQB1* 02 allele.” Autoimmunity 35.5 (2002): 365-368.

Damci, T., et al. “Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in Type 1 diabetic patients.” European journal of clinical investigation 33.5 (2003): 397-401.

Support for expression of tight junctions

- Prebiotics for buryrate production or butyrate (coated)
- probiotics
- Glutamine
- Green Tea, Resveratrol, Curcumin
- Vit D3
- Gamma-Lineolic Acid (GLA)

Bischoff, Stephan C., et al. “Intestinal permeability–a new target for disease prevention and therapy.” BMC gastroenterology 14.1 (2014): 189.

Kuitunen, Mikael, et al. “Intestinal permeability to mannitol and lactulose in children with type 1 diabetes with the HLA-DQB1* 02 allele.” Autoimmunity 35.5 (2002): 365-368.

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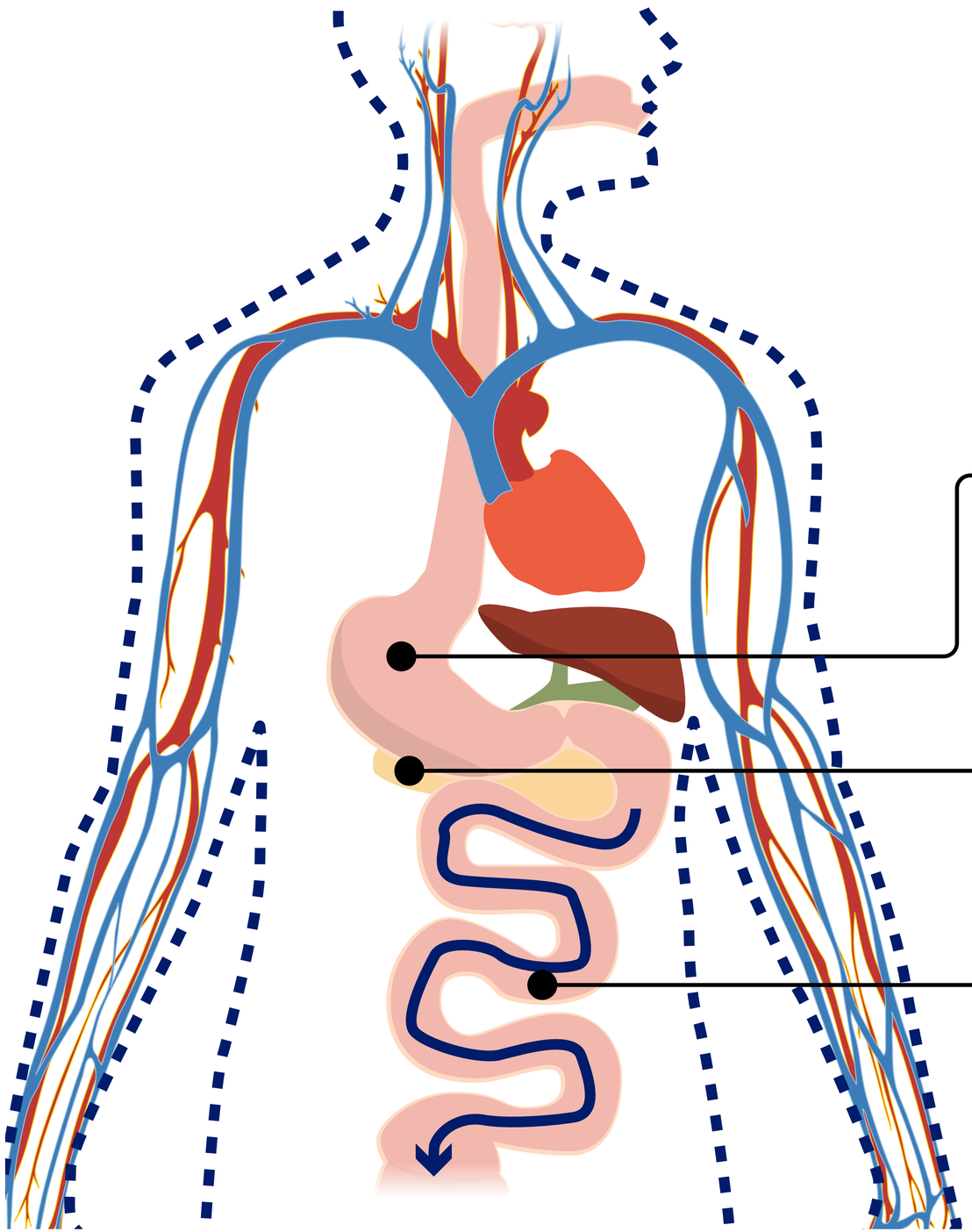

Global intestinal is a multilevel support

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 juction optimazing (pH 6-7)
- ↓inflammation
- Improve the synthesis of s IgA by the intestinal lymphocytes

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

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Personalized advise for practitioners

register as healthcare professional on academy@nutrined.nl