Understanding the Role of Spore based Bacteriotherapy in the Treatment of Chronic Infection and Immune Dysfunctions

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AARM

Association for the Advancement of Restorative Medicine

Disclosure Statement

- Kiran Krishnan is the acting Chief Science Officer for Microbiome Labs, a provider of novel probiotic products to physicians.
- This educational activity has been reviewed by the California Naturopathic Doctors Association and contains no commercially biased information

A NEW PERSPECTIVE:

HUMAN IS MERELY A COLLECTION OF SYMBIONTS OR BIONTS WHO FORM A HOLOBIONT – A SUPER ORGANISM

"Symbiogenesis is the result of the permanent coexistence of various bionts to form the holobiont (namely, the host and its microbiota"

Ricardo Guerrero et al, Aug 2013

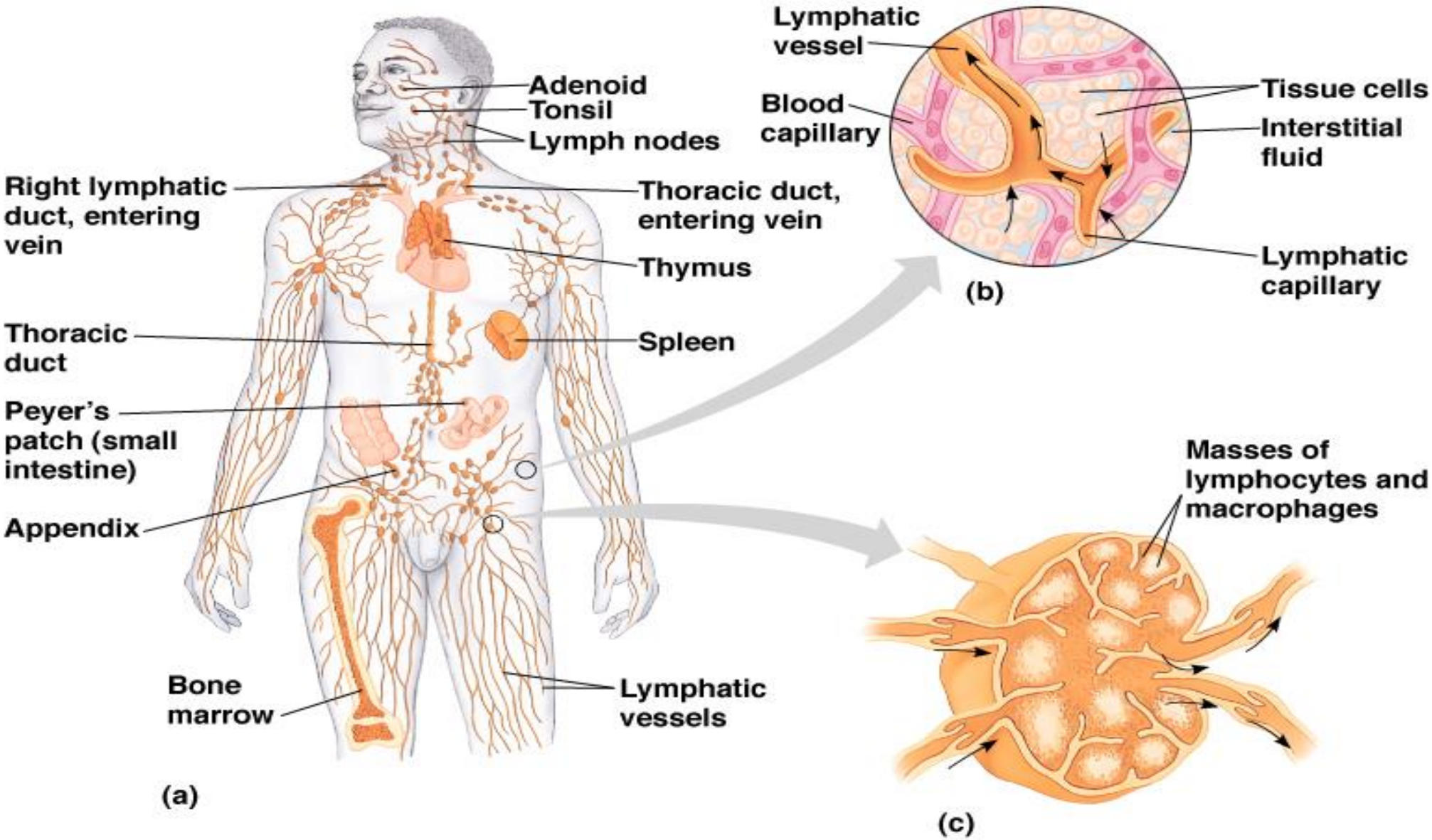
"Eukaryotic individuals can be analyzed as coevolved, tightly integrated, prokaryotic communities; in this view, natural selection acts on the holobiont as if it were an integrated unit."

Ricardo Guerrero et al, Aug 2013

THE IMMUNE SYSTEM

- Human immune system begins to develop in the embryo.
- Starts with hematopoietic (from Greek, "blood-making") stem cells.
- Stem cells differentiate into major cells in the immune system
  - granulocytes, monocytes, and lymphocytes
- The only major system in the body designed to protect us
- Immune system is an army with no general – requires training.
- Takes at least 6 months for the immune system to start working on its own
- Stem cells continue to be produced and differentiate throughout ones lifetime.

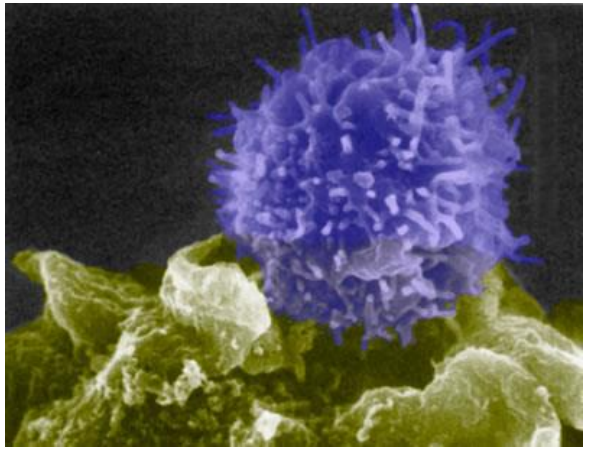
COMPONENTS OF THE HUMAN IMMUNE SYSTEM



Immunity and Immune Response

Made up of two cellular systems:

- **Humoral or circulating antibody system**
  - **B cells produced antibodies**



- **Cell mediated immunity**
  - **T cells primarily**

INNATE VS. ADAPTIVE IMMUNITY

INNATE IMMUNITY (our first line of cellular defense)

➢ **ANTIGEN PRESENTING CELLS** – Macrophages and Dendritic cells – find and present potential problems.

- They sit amongst trillions of bacteria in the mucosal system and have to actively recognize friend and foe!
- These APCs are produced by the thymus and other lymphoid centers but are recruited to the gut mucosa by our commensal organisms.
- The microbiome helps these cells by expressing something called Toll-like receptors (TLRs) – These TLRs neutralizes immune response to offer “tolerance”.
- The microbiome also goes as far as producing ATP (energy) to help these cells differentiate and function.

➢ **NEUTROPHILS** – Key part of first line of defense

- Killer cells that directly target harmful organisms. Very important to maintain infection free in the cold and flu season.
- Dependent on the microbiota to stimulate their expression and even to equipt them with the tools to perform their killing function – nitric oxide, super oxides, etc.

INNATE IMMUNITY (our first line of cellular defense)

- **NATURAL KILLER CELLS –** Highly important in viral infections. These cells identify infected tissue and eliminates it. With dysfunction in NK cells, an individual would face chronic, consistent infections.
  - The microbiota stimulates the production of NK cells.
  - The microbiota effects the potency of the cells as well.

- **MAST CELLS –** Highly important regulatory cells in the lamina propia.
  - They control blood flow and coagulation in the LP.
  - They control smooth muscle cell peristalsis.
  - Fight agains gut permeability
  - Control electrolyte exchange
  - Poor microbiota and low diversity leads to fewer mast cells in the gut and more in circulation – one mode of action for increasing allergies.

- **INTESTINAL EPITHELIAL CELLS (IEC)** – The barrier cells that have some immune function
  - Releases key antimicrobials to protect the barrier
  - Releases chemokines and cytokines to recruit immune cells to the location
  - The microbiota stimulates the IEC to release these antimicrobials and chemical messengers.

INNATE VS. ADAPTIVE IMMUNITY

ADAPTIVE IMMUNITY – The second line of defense and the long term protection

B-Cells: (antibody secreting cells)

- Gut associate B-cells primarily secret IgA – this is the antibody that is made in the highest concentration and we make about 7g of it each day!
- B-cells originate in the Peyers patches
- The amount of B-cells/Peyers patches and their potency is directly controlled by commensal bacteria.
- IgA, unlike IgM, has low “memory” and mostly recognizes current crop of commensals and invading organism. It requires constant stimulation and up-regulation to provide new IDs and protection.
- Low microbiota diversity, low microbial exposure, low antigenic species in our environment leads to low levels of IgA production and actually higher IgE production!

INNATE VS. ADAPTIVE IMMUNITY

ADAPTIVE IMMUNITY – The second line of defense and the long term protection

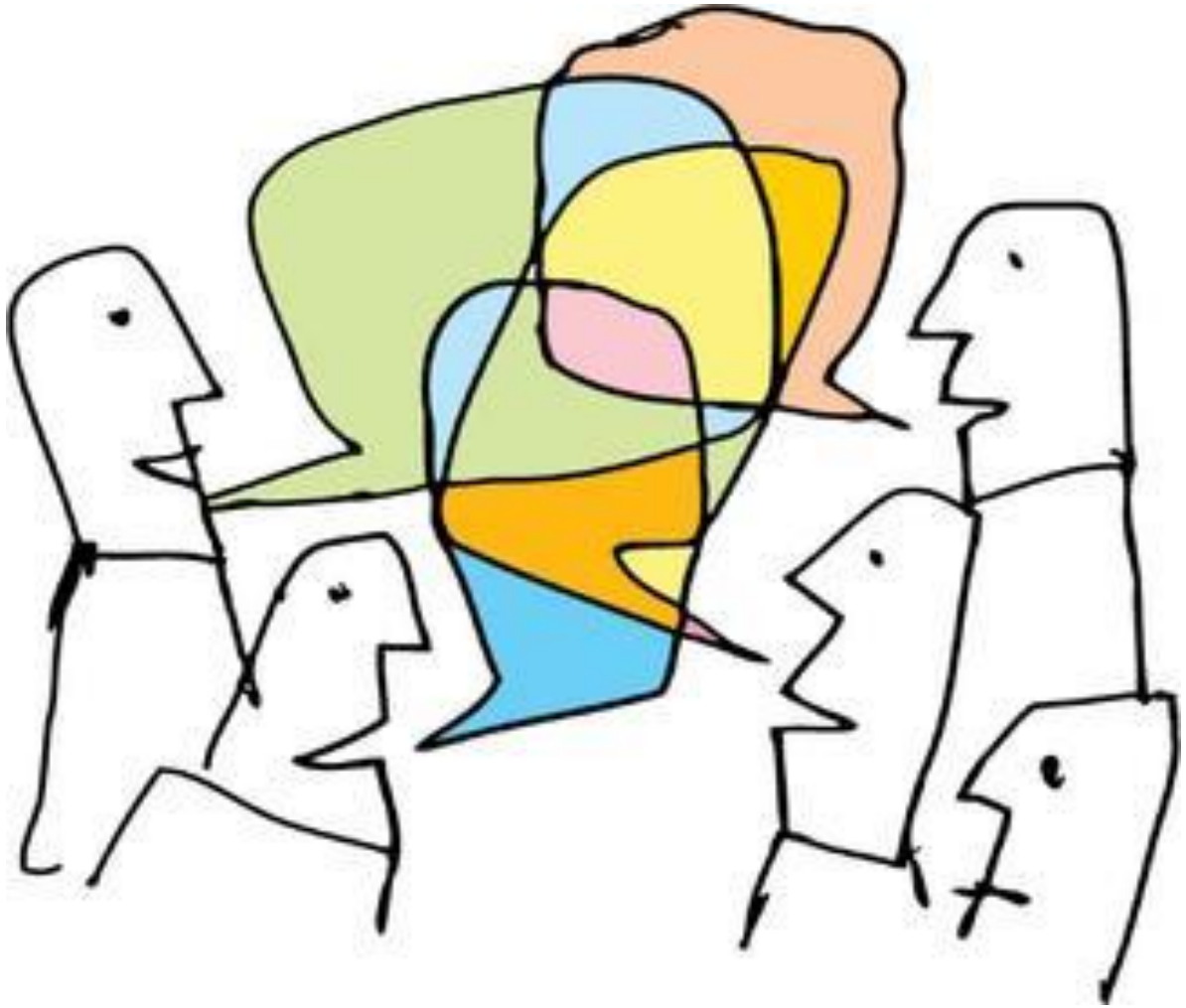
T-Cells – (Our Immune Orchestrators)

CD4+ T cells are the T cells that can differentiate into Th1, Th2, Th17 or Treg cells.

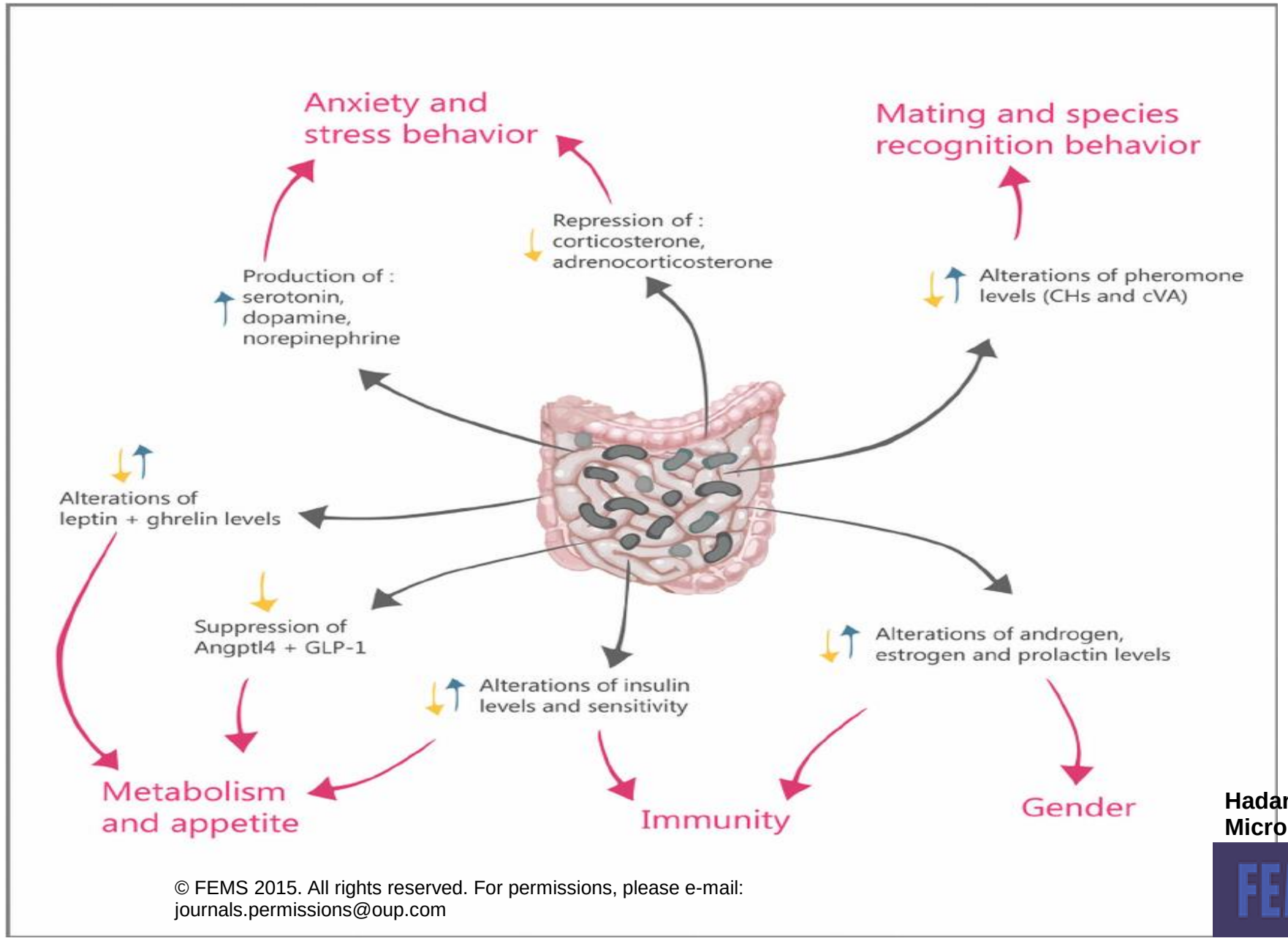
HAVING BALANCE IN THESE 4 SUB-TYPES IS CRITICAL TO HEALTH

- Th1 protects against intracellular microbial infections
- Th2 protects against parasites
- Th17 is pro-inflammatory and acts in the heat of battle
- Uncontrolled Th expression causes disease: Too much Th1 and Th17 is linked to autoimmune conditions. Too much Th2 is linked to allergic and sensitivity reactions.
- Treg regulates the balance and favors tolerance. When Treg expressions are low, it leads to autoimmune conditions and severe allergies
- A weak microbiome leads to Th1/Th2 imbalance and typically leans towards Th2
- The microbiota is responsible for stimulation and maturation of Tregs, when the microbiota is weak we see increased colitis risk. We find a low level of colonic Treg cells and so T-cells in the colon attack the tissue and commensals.

CROSS-TALK AND COMMUNICATION BETWEEN THE MICROBIOME AND THE HOST COMPONENTS



MICROBIAL ENDOCRINE SYSTEM

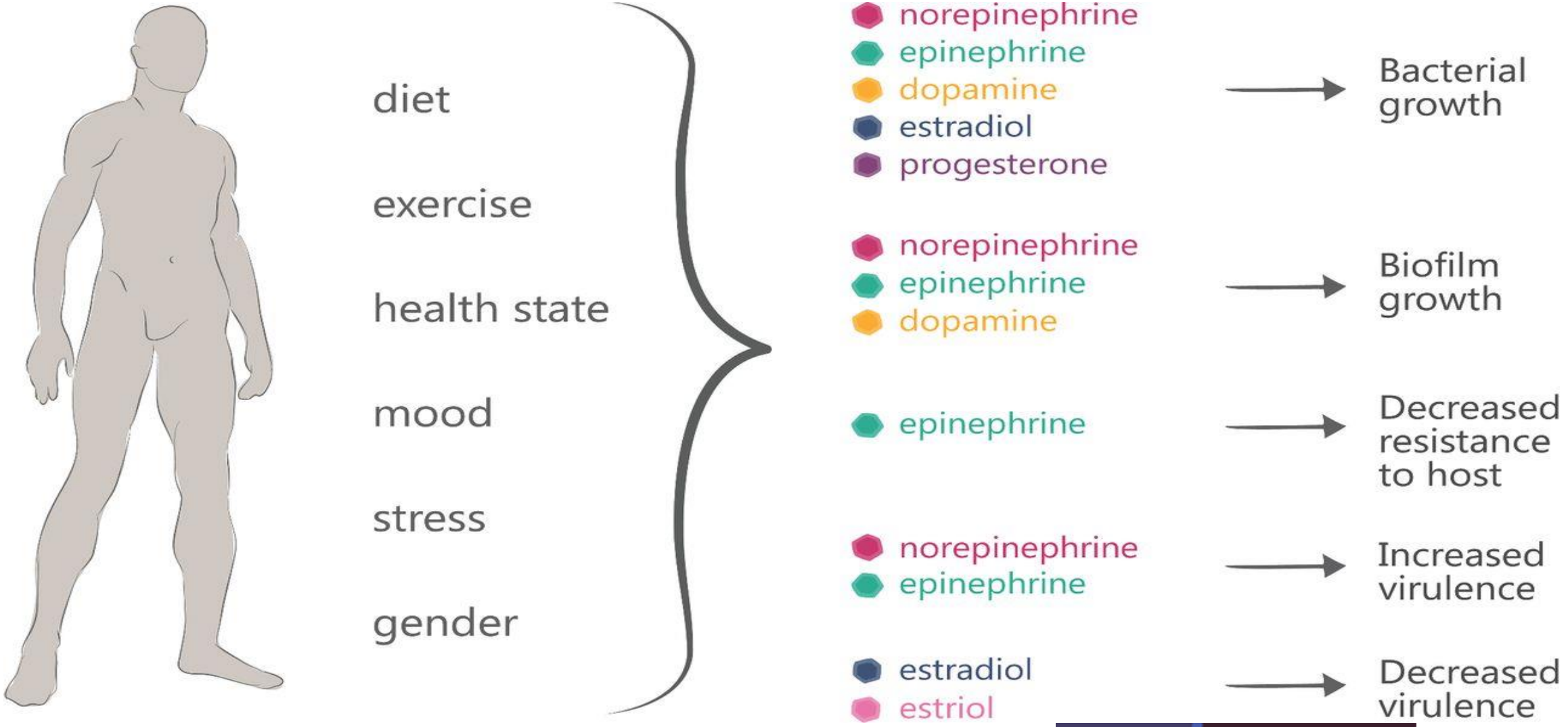


Hadar Neuman et al. FEMS Microbiol Rev 2015;femsre.fuu010

FEMS MICROBIOLOGY REVIEWS

MICROBIAL ENDOCRINE SYSTEM

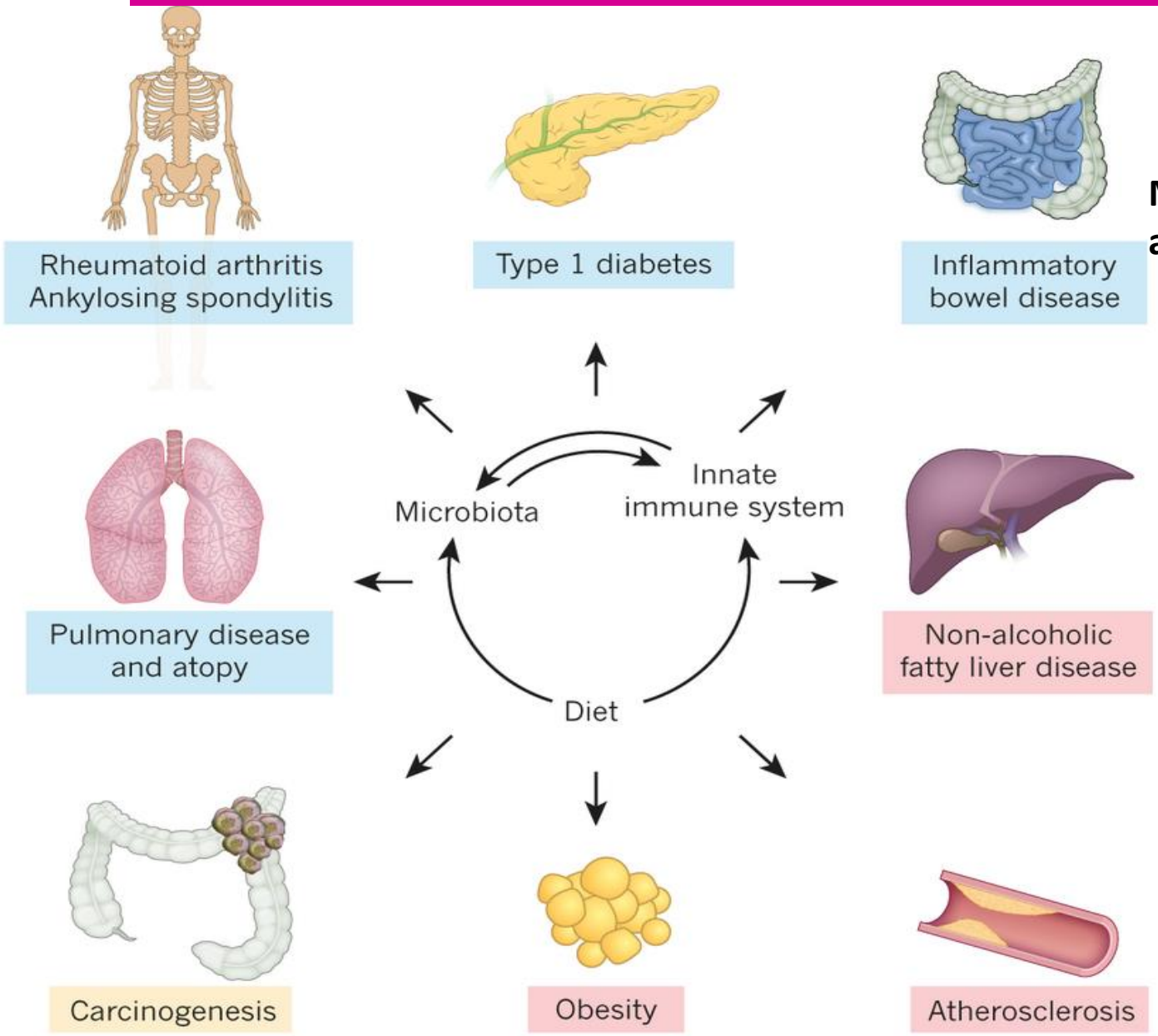
Host effects on the microbiota.



Hadar Neuman et al. FEMS Microbiol Rev 2015;femsre.fuu010

FEMS MICROBIOLOGY REVIEWS

MICROBIOME AND THE INNATE IMMUNE SYSTEM



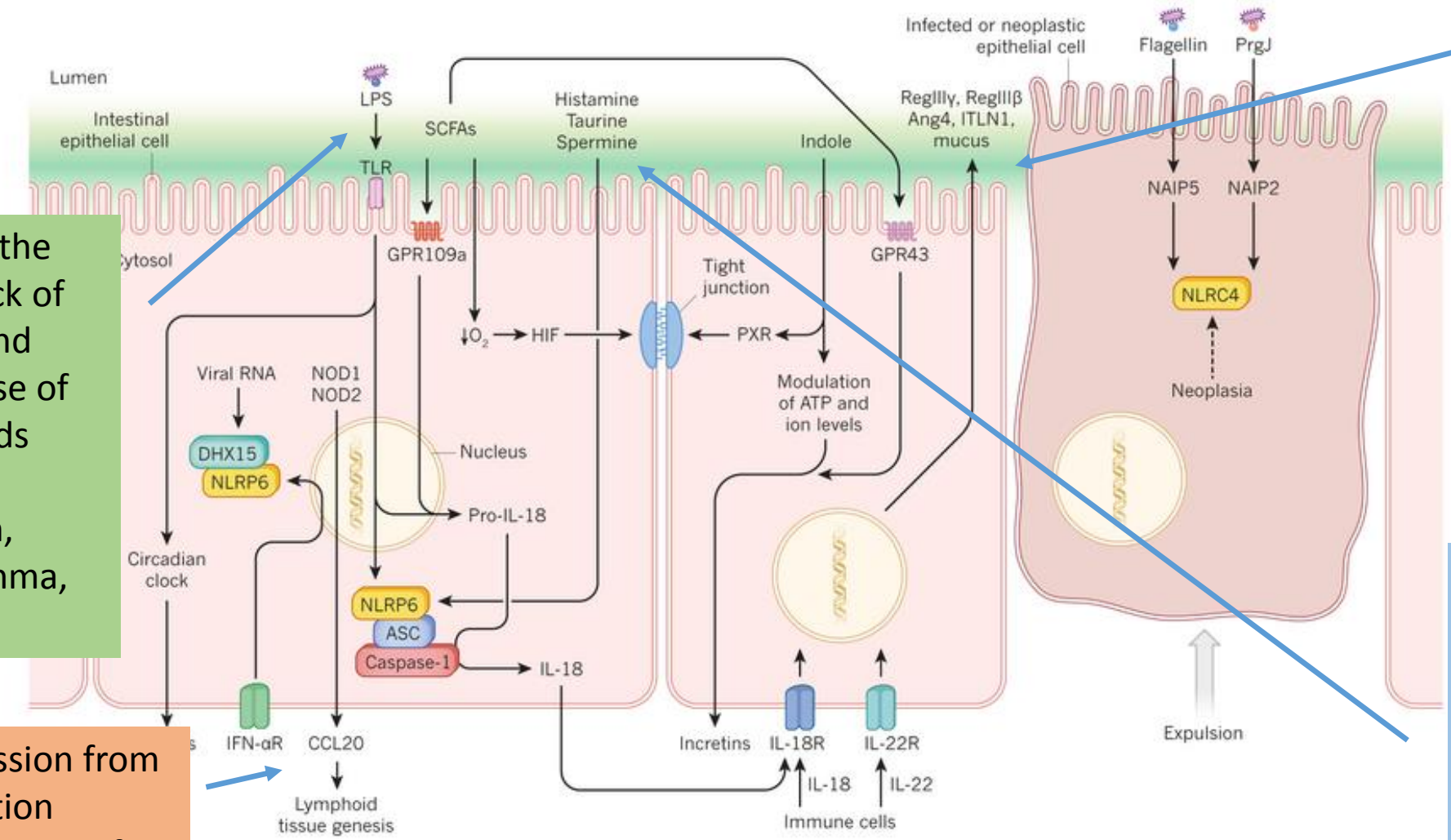
Microbiome–innate-immune-system interactions are involved in multifactorial diseases.

Many inflammatory disorders are influenced by alterations in the crosstalk between innate immunity and the microbiome.

Modulation of the severity of a disorder through dietary interventions and their influence on microbiome–immune interactions is an exciting area of research.

Nature **535**, 65–74 (07 July 2016) **doi:10.1038/nature18847**

MICROBIOME AND THE INNATE IMMUNE SYSTEM



Intestinal epithelial cells orchestrate the host–microbiota interface.

PPRs control the circadian clock of epithelium and control release of glucocorticoids which fights inflammation, allergies, asthma, etc.

PPRs/TLRs and NOD expression from gut commensals stimulates the release of antimicrobial peptides from epithelium

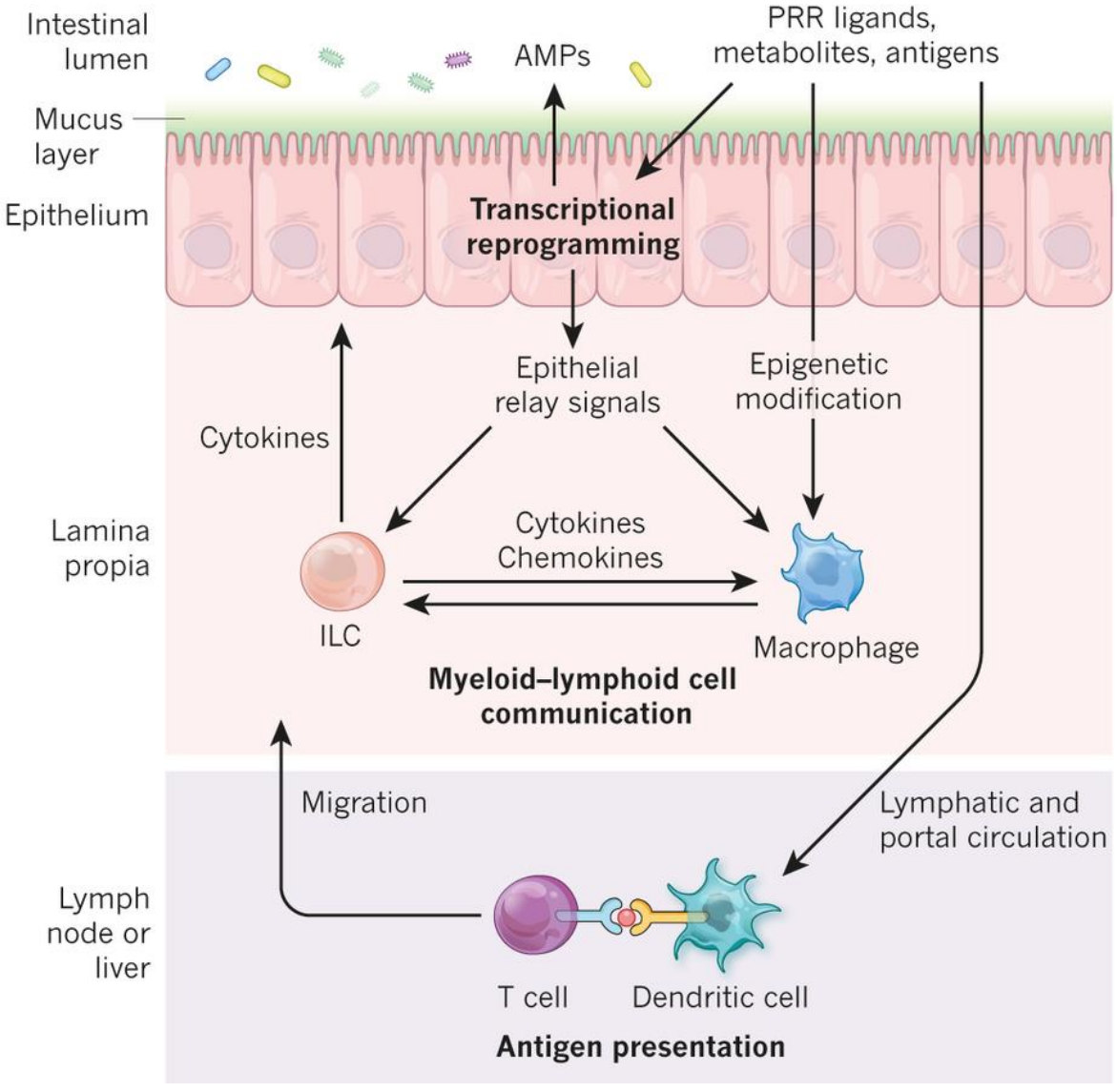
Histamine, taurine and indole from the microbiome stimulate NLRP6 and Type 1 interferon which act as viral detectors

NOD 1 expression from CCL20 activation stimulates genesis of lymphoid tissue

Nature **535**, 65–74 (07 July 2016) **doi:10.1038/nature18847**

MICROBIOME AND THE INNATE IMMUNE SYSTEM

The hierarchy of anatomy in microbiome–innate-immune-system interactions.



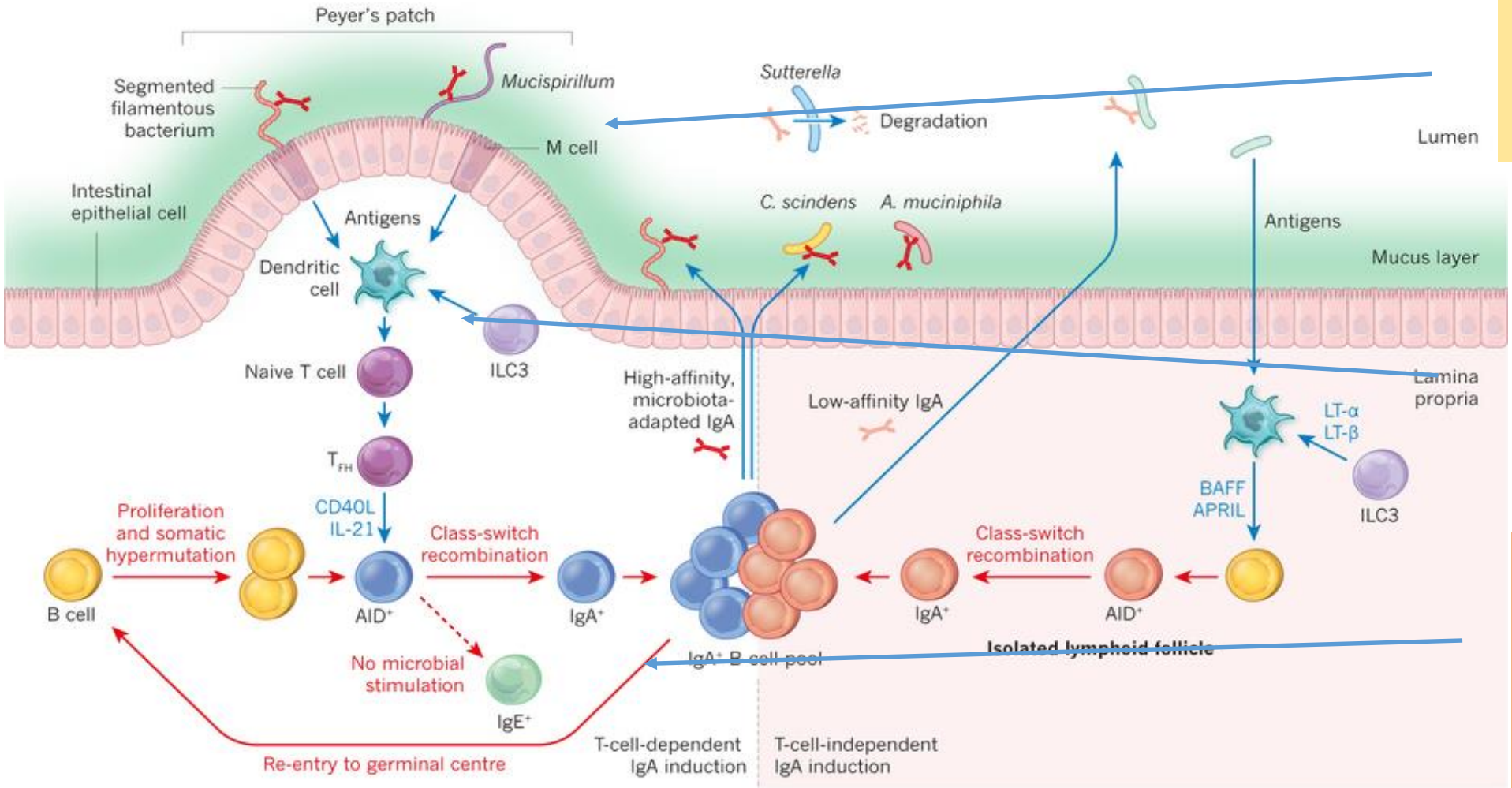
Microbiome influence on the innate immune system works via feedback loops. IL-18 is a prototypical communication cytokine for this system.

The impact of the microbiome loops into the lamina propia through PRR ligands, metabolites and antigens. Some commensal bacteria components can even reach the lymph node and cause an activation of dendritic cells that activate anticommensal-T cells to promote microbial containment.

Nature 53565–74(07 July 2016) doi:10.1038/nature18847

MICROBIOME AND THE ADAPTIVE IMMUNE SYSTEM

The microbiota in adaptive immune homeostasis and disease



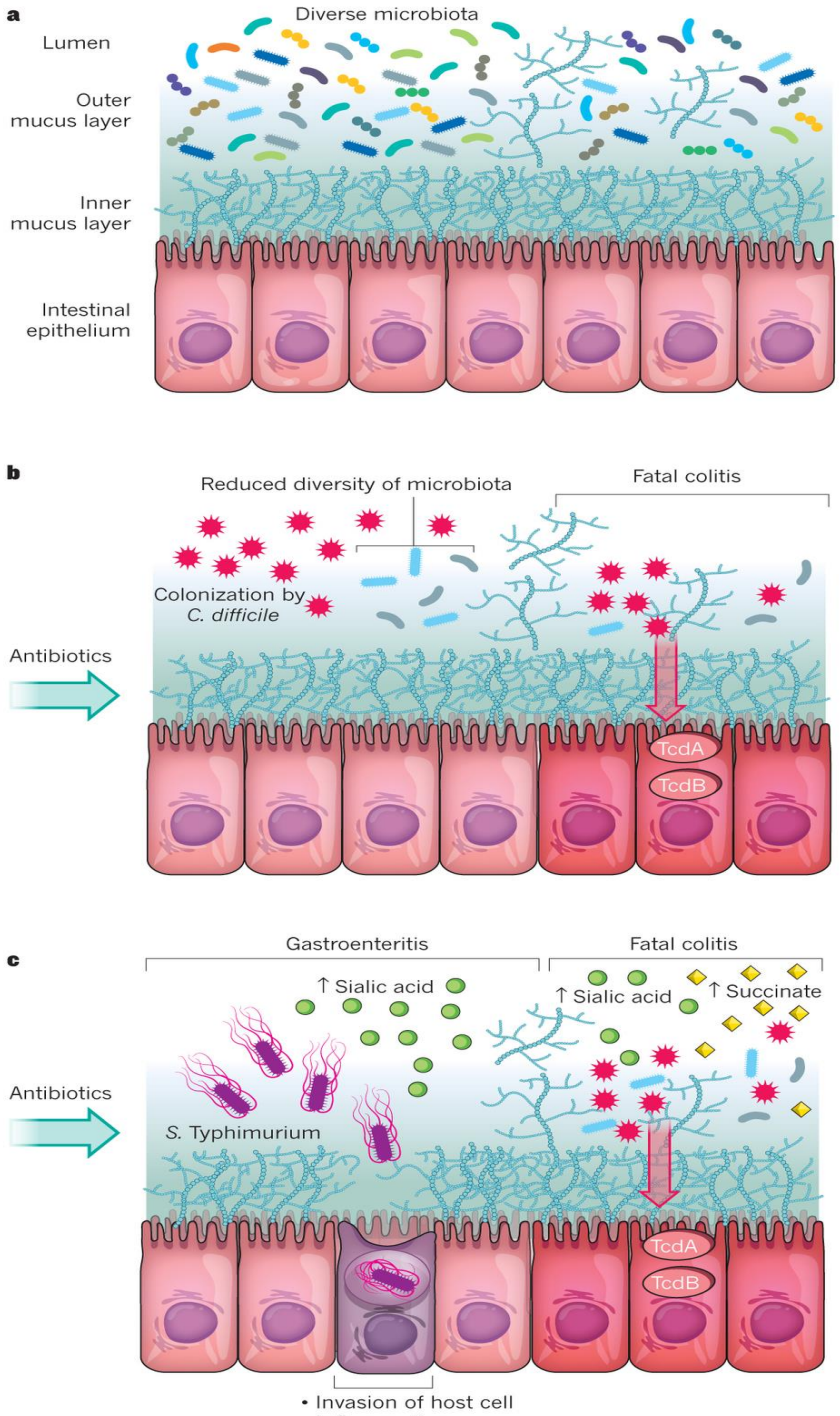
Microbiome cells or their antigens bind to the epithelium alerting M Cells

These cellular components of antigens are then presented to dendritic cells in the lamina propia.

Activated Dendritic cells then differentiate CD4+ T cells to T Follicular helper cells. T FS cells active B cells which causes IgA release

This process produces both high affinity and low affinity IgA to protect the host and allow for adaptation with a changing microbiome.

Nature 535, 75–84 (07 July 2016) doi:10.1038/natu

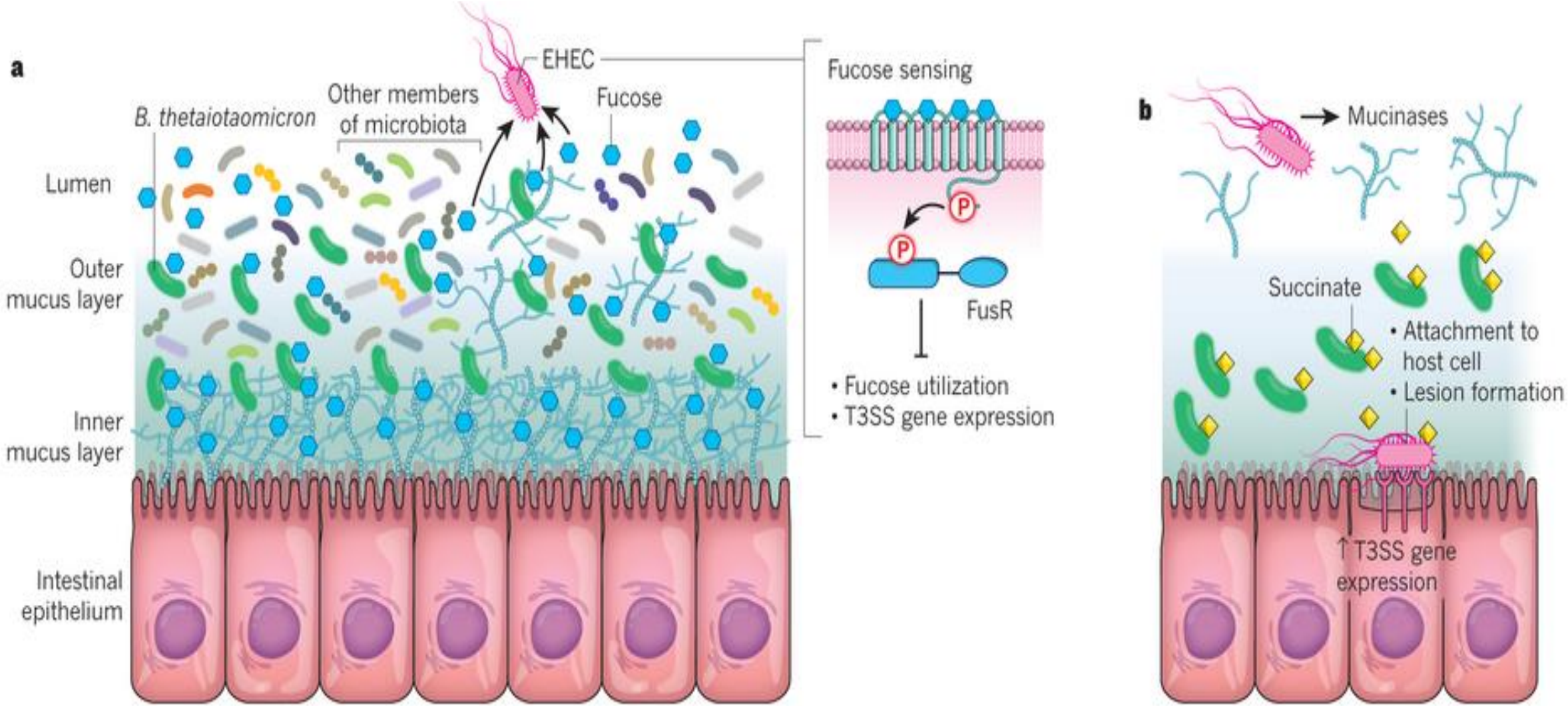


The impact of antibiotics on the microbiota and the expansion of enteric pathogens.

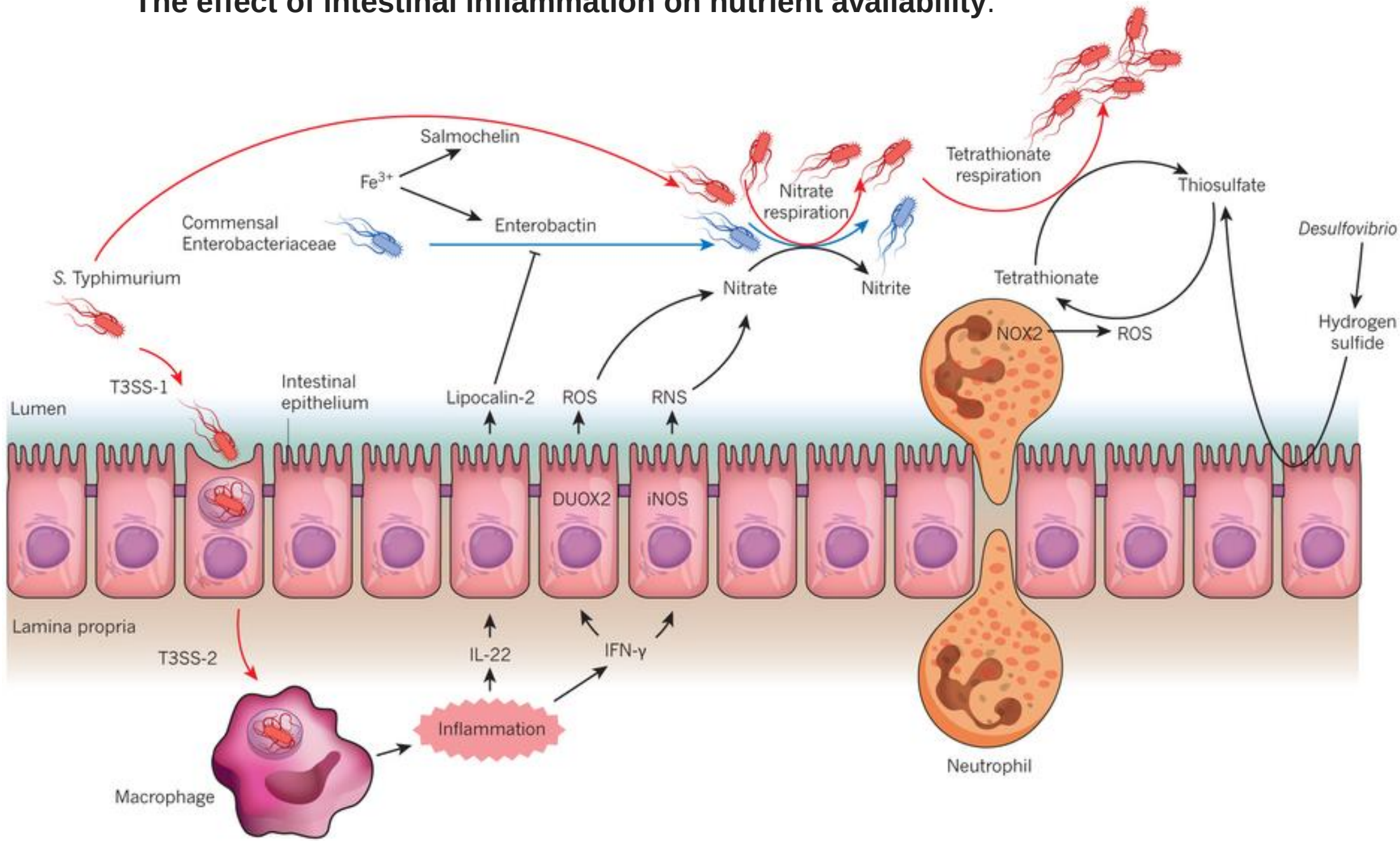
a, A diverse and non-disturbed microbiota confers resistance to colonization by enteric pathogens in the intestinal epithelium. **b**, Treatment with antibiotics decreases the diversity of the microbiota and leads to expansion of the *C. difficile* population. Toxins that are released from *C. difficile* (TcdA and TcdB) enter and damage the cells of the epithelium, which leads to inflammation (colitis) and cell death. **c**, Treatment with antibiotics also leads to an increase in the levels of free sialic acid (from the host) and succinate (from the microbiota) in the lumen of the intestine. Elevated sialic acid promotes the expansion of the *S*. Typhimurium population, which can lead to inflammation (gastroenteritis) if the bacterium invades the cells of the intestinal epithelium. Elevated levels of sialic acid and succinate further promote the expansion of the *C. difficile* population and the development of colitis and cell death.

Nature
535,
85–93
(07 July 2016)
doi:10.1038/nature18849

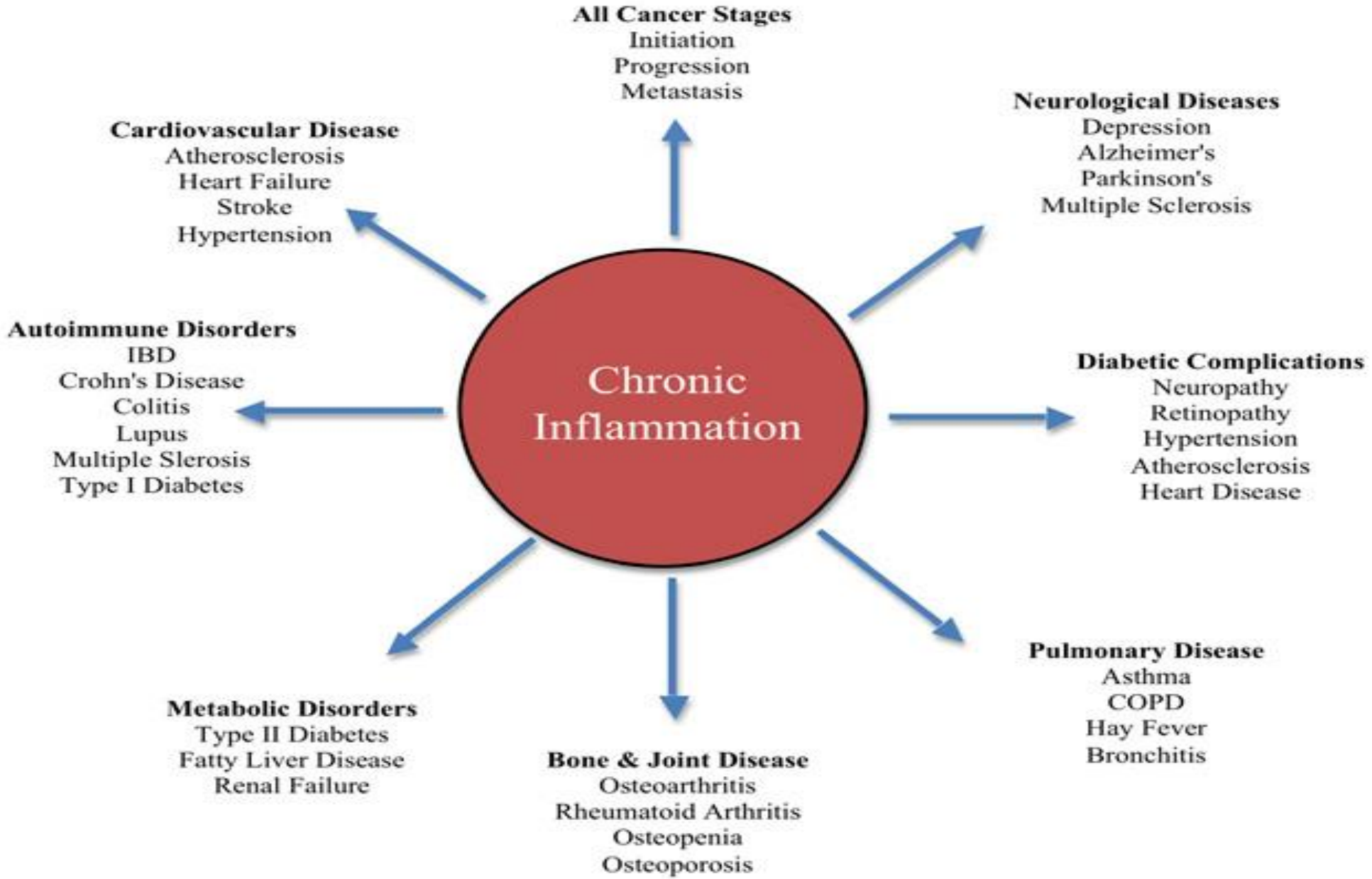
Modulation of enterohaemorrhagic E.coli virulence through nutrients provided by the microbiota.



The effect of intestinal inflammation on nutrient availability.



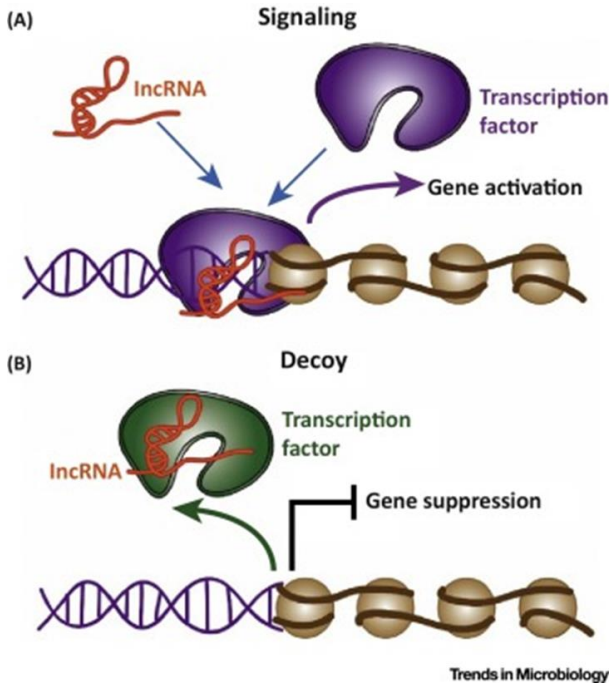
THE BOTTOM LINE

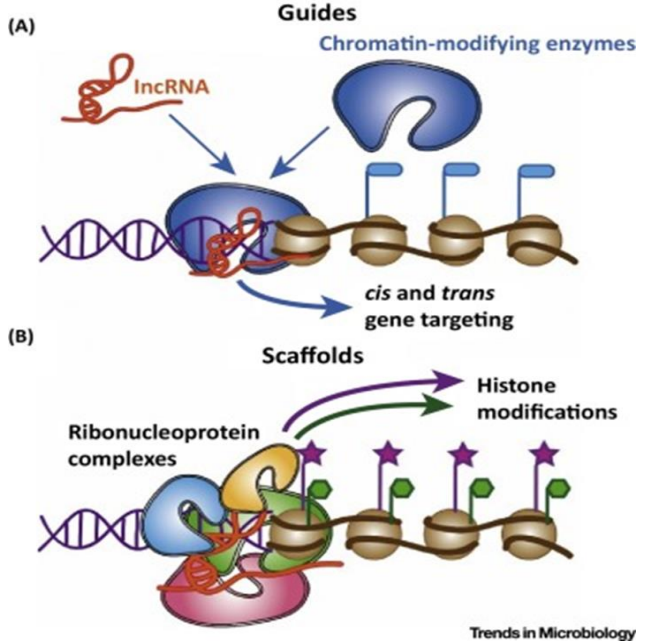


MICROBIAL CONTROL OF EUKARYOTIC CELL EXPRESSION

OUTER MEMBRANE VESICLES AND HUMAN EXOSOMES:

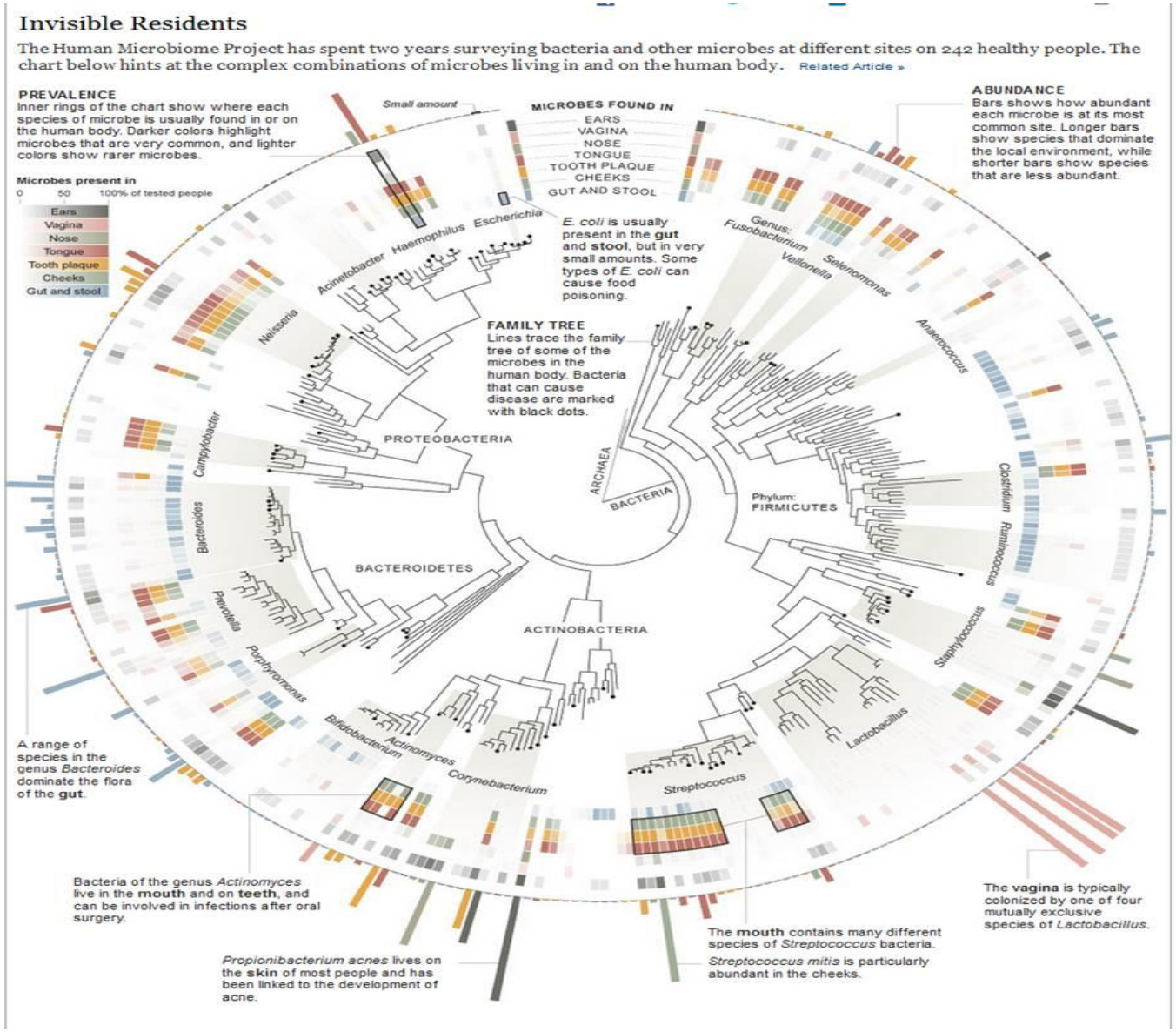
- OMVs are bacterial produced excretory nanoparticles that can be tissue trophic
- OMVs can contain peptides, neurotransmitter and microRNA – microRNA is best studied
- Exosomes are human epithelia cell produced extracellular nanoparticles that effect the microbiome.
- This is a display of inter-kingdom communication and influence
- OMVs can be produced by viruses, fungi and even our food!





Methylation and Phosphorylation control

WHAT IS A HEALTHY MICROBIOME?



A DIVERSE MICROBIOME!

Diversity Gives Strength:

1) Can do more functions
2) More functional redundancies
3) Diverse communities are more resistant to invasion

What Effects The Microbiome?

1) Age
2) Diet
3) Antibiotic Use
4) Physiology
5) Genetics - loosely

The Importance of Microbiome Diversity

"Using comparative microbiome profiling of a cohort of CRS (chronic rhinosinusitis) patients and healthy subjects, we demonstrate that the sinus microbiota of ***CRS patients exhibits significantly reduced bacterial diversity*** compared with that of healthy controls."

Abreu NA, Nagalingam NA, Song Y, et al. ***Sinus Microbiome Diversity Depletion and Corynebacterium tuberculostearicum Enrichment Mediates Rhinosinusitis.*** *Science translational medicine*. 2012;4(151):151ra124. doi:10.1126/scitranslmed.3003783.

"***High diversity*** has been generally associated with health and temporal stability"

"Conversely, ***a relative lack of diversity*** is apparent in the gut microbiome in diseases ranging from obesity to inflammatory bowel disease and types 1 and 2 diabetes; and in the skin microbiome in atopic dermatitis and psoriasis."

Lloyd-Price J, Abu-Ali G, Huttenhower C. ***The healthy human microbiome.*** *Genome Medicine*. 2016;8:51. doi:10.1186/s13073-016-0307-y.

INTERMITTENT FASTING

Cell Metab. 2014 Dec 2;20(6):1006-17. doi: 10.1016/j.cmet.2014.11.008.

Diet and feeding pattern affect the diurnal dynamics of the gut microbiome.

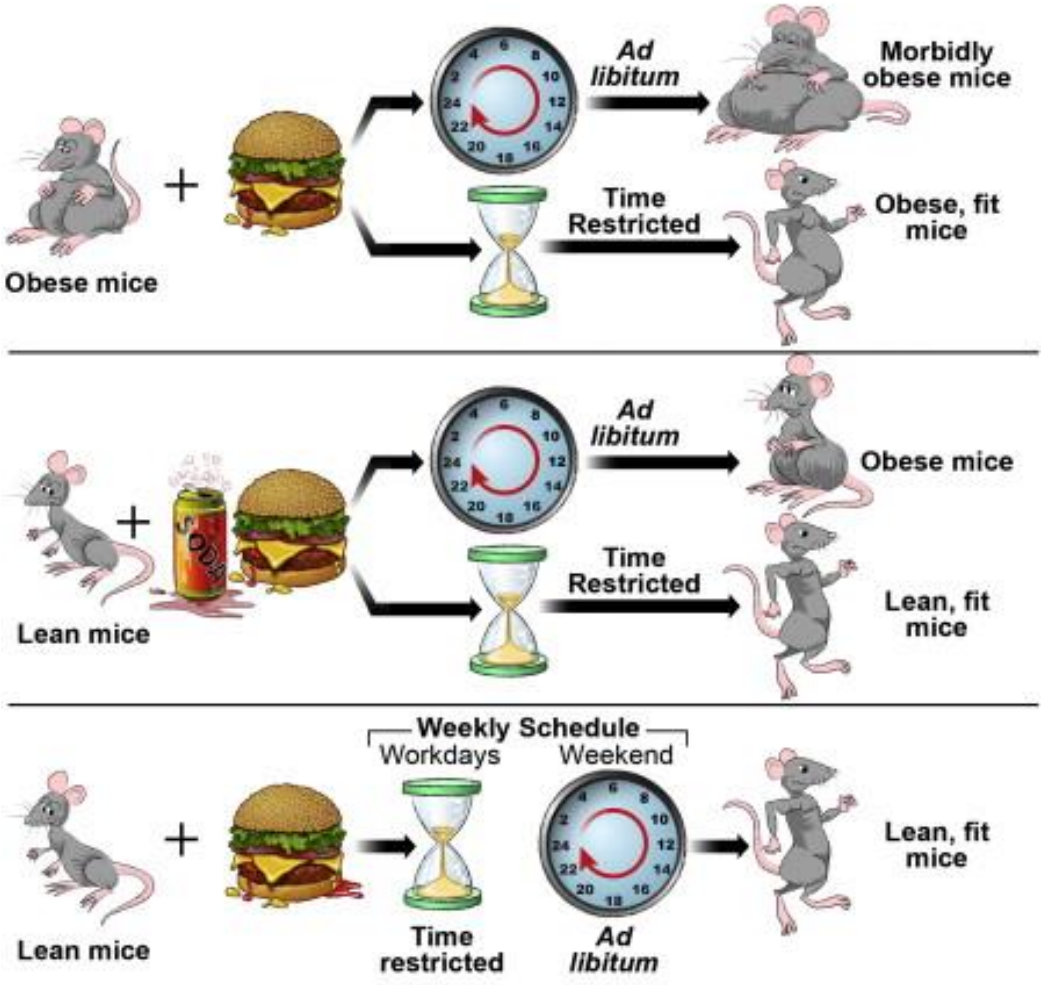
Zarrinpar A[1], Chaix A[2], Yooseph S[3], Panda S[4].

Abstract

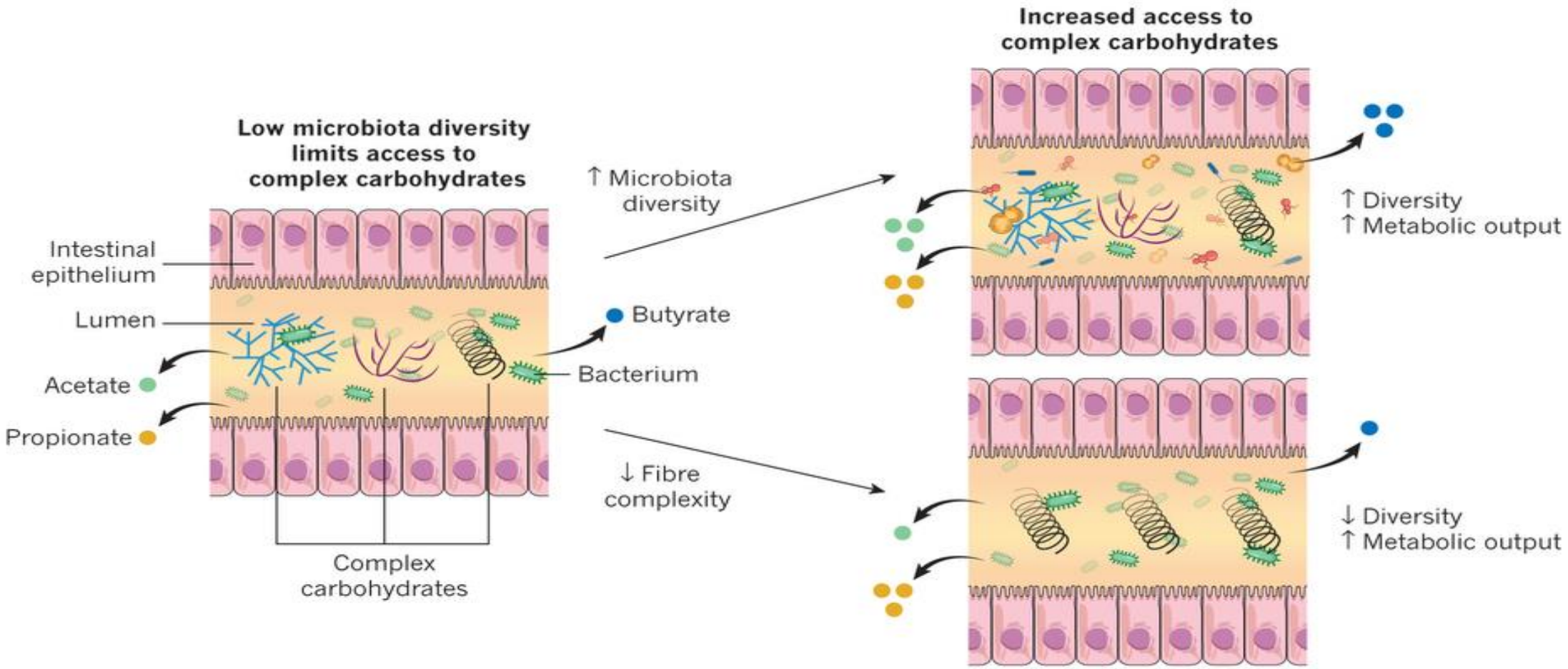
The gut microbiome and daily feeding/fasting cycle influence host metabolism and contribute to obesity and metabolic diseases. However, fundamental characteristics of this relationship between the feeding/fasting cycle and the gut microbiome are unknown. **Our studies show that the gut microbiome is highly dynamic, exhibiting daily cyclical fluctuations in composition.** Diet-induced obesity dampens the daily feeding/fasting rhythm and diminishes many of these cyclical fluctuations. **Time-restricted feeding (TRF), in which feeding is consolidated to the nocturnal phase, partially restores these cyclical fluctuations. Furthermore, TRF, which protects against obesity and metabolic diseases, affects bacteria shown to influence host metabolism. Cyclical changes in the gut microbiome from feeding/fasting rhythms contribute to the diversity of gut microflora and likely represent a mechanism by which the gut microbiome affects host metabolism.** Thus, feeding pattern and time of harvest, in addition to diet, are important parameters when assessing the microbiome's contribution to host metabolism.

INTERMITTENT FASTING

Time-Restricted Feeding Is a Preventative and Therapeutic Intervention against Diverse Nutritional Challenges. Cell Metabolism

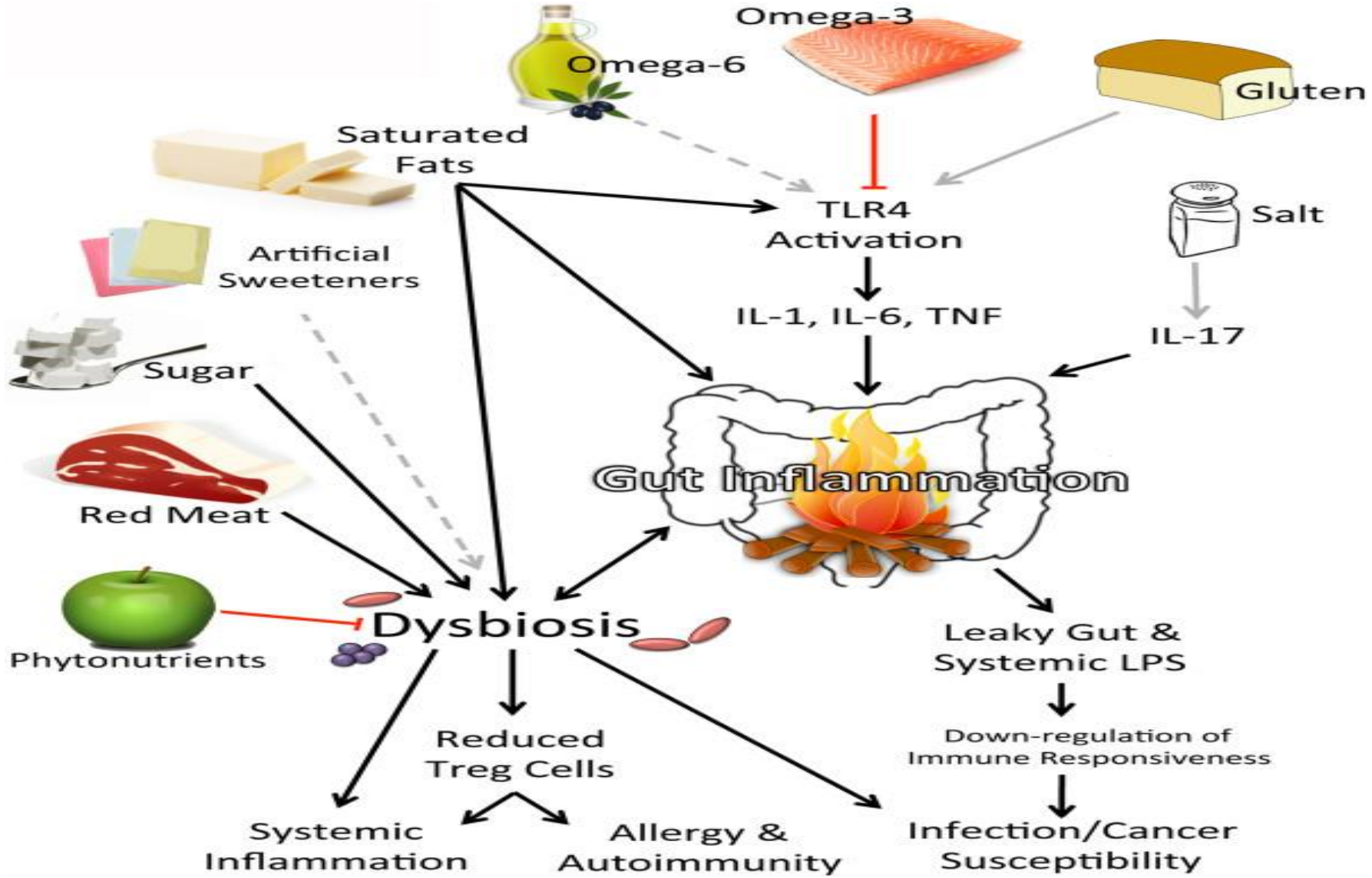


Interactions between the diet and the gut microbiota dictate the production of short-chain fatty acids

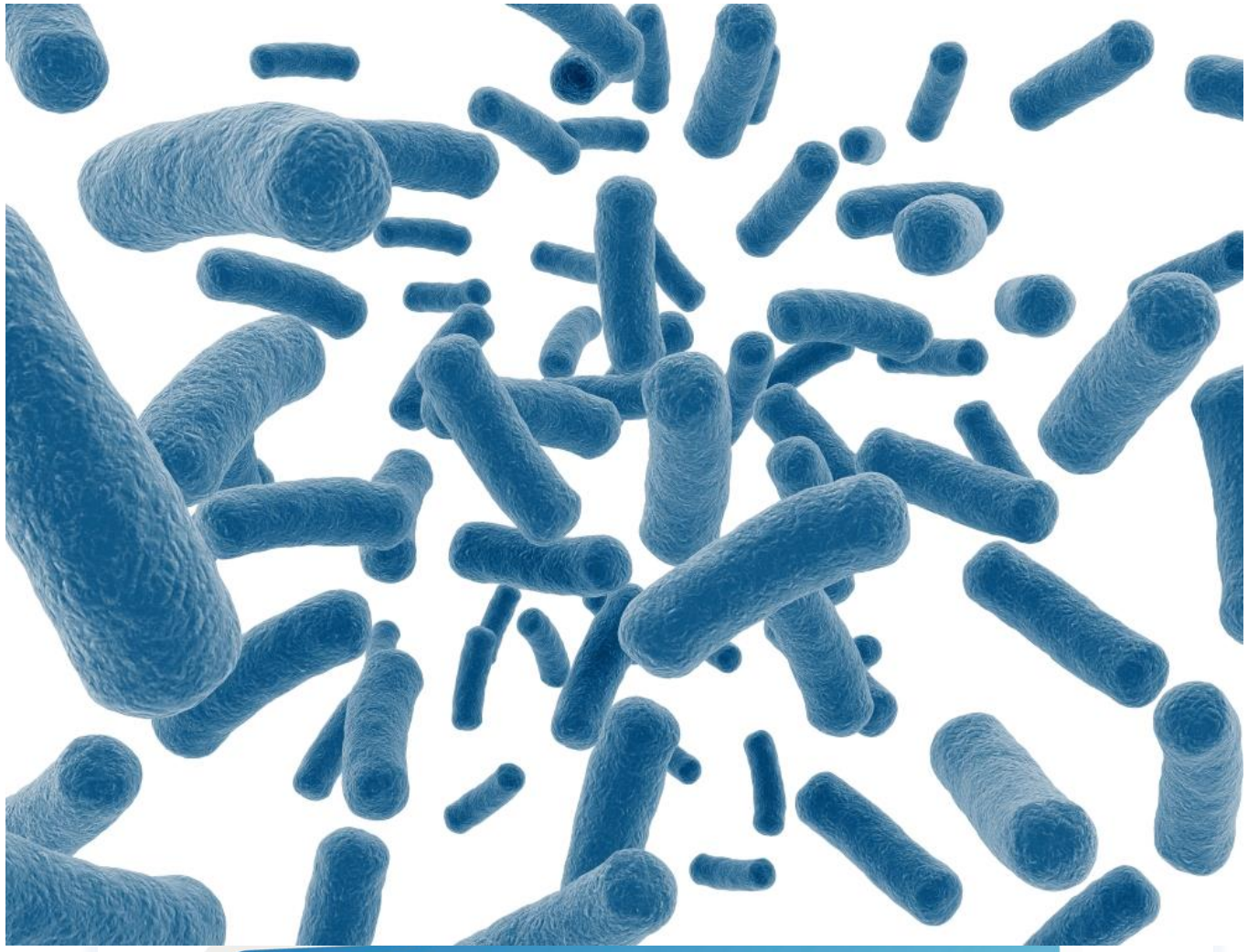


Nature 535, 56–64 (07 July 2016) doi:10.1038/nature18846

ALTERATIONS OF THE MICROBIOME FROM DIET



Nutrition Journal Volume 13, Number 61, doi: 10.1186/1475-2891-13-61



PROBIOTICS

SURVIVAL

The Food Standards Agency (FSA) study w/ Reading University (UK)

Dr. G.R. Gibson, Dr. G. Rouzaud, Dr. J. Brostoff and Dr. N. Rayment

Purpose:

1) Evaluate the probiotic effect of commercial products in the human gut. Any affect on gut flora.
2) 35 strains from commercial products were studied. Primarily *lactobacillus sp.* and *bifidobacterium sp.*
3) Evaluate the survivability of common probiotics through the GIT.

STEP 1: Survival through gastric juices in the stomach. 20 minutes at pH 1-3

pH 1 = None survived with any viability. Unable to recovery

pH 2-3 = 18 of the 35 showed around 50% survival

STEP 2: Survivors through the stomach were tested for survival in the upper small intestines via bile acid tolerance

Of the 18 tested, only 6 showed viability in the presence of bile acid salts and would have a chance to make it to the large intestines.

STEP 3: The 6 strains were then chosen for survival testing in lower intestines

Of the 6 strains, 4 strains showed viability in lower intestinal conditions

=

Only 4 of 35 strains would survive to enter the large intestine and the survivors would have less than 50% survival

ISSUES - THE RESEEDING PLAN

FITNESS:

- Aerobic bacteria seeding an Anaerobic Environment
- Evade immune destruction
- Do they fit in your gut – binding sites

Larsen, N et al (2009). A comparative study on adhesion and recovery of potential probiotic strains of Lactobacillus spp

Included popular strains such as; L. rhamnosus GG, L. reuteri ATCC 55730, L. johnsonii NCC 533 and L. reuteri DSM 12246.

Results: The best adhesion was 38% by L. reuteri, second best was 24% and the rest went down from there.

IS IT NATURAL:

- Does it happen in nature?
- The issue of Finite Overload – goes against expanded diversity

THE IMPORTANCE OF ENVIROMENTAL BACTERIA

- **Distinct Distal Gut Microbiome Diversity and Composition in Healthy Children from Bangladesh and the United States**
  Audrie Lin, et al 2013

"The distal gut of Bangladeshi children harbored significantly greater bacterial diversity than that of U.S. children, including novel lineages from several bacterial phyla."

- **Human gut microbiota community structures in urban and rural populations in Russia**
  Alexander V, et al 2013

"the original microbial community structures occurred in hosts from urban populations 2.6-fold less frequently than in the rural hosts, which implies that the rural population's microbiota community was the healthy original"

- **Comparison of fecal microflora of elderly persons in rural and urban areas of Japan.**
  Benno Y, et al 1989

'found significant rural-urban disparities in microbiota composition. Rural populations had much higher bifidobacteria levels…"

THE IMPORTANCE OF ENVIROMENTAL BACTERIA

STUDIES ON THE HAZDA TRIBE OF TANZANIA

- Some of the last hunter-gatherer people on earth
- They live an ancient, ancestral life.
- Their environment hasn't changed for 1000s of years.
- Massive exposure to ancestral microbial community
- Vastly different microbiota compared to westernized populations
- Virtually no common digestive diseases such as Crohn's, UC, Colon Cancer, Reflux, etc.

REQUIRED FEATURES OF NATURE'S PROBIOTIC

- **Naturally survive the harsh gastric environment**
- **Be of a strain that is found in the microbiota** – It has to have a binding site and belong in the gut.
- **Must have evolutionary significance**
- **Must be a facultative anaerobe**
- **Must have a bi-phasic life cycle**
- **Must have clinical demonstration of safety and efficacy**

Bacterial Spores!

- In particular *Bacillus* spores as they are the most widely studied and most widely used probiotics outside of the supplement market.
- *Bacillus* spores were the first commercial probiotics. Were also the first prescription probiotics starting in 1958:
  - Enterogermina® (Sanofi-Aventis, Italy)
  - Bacti-Subtil® (Aventis Pharma, France)
- Used extensively in agriculture and aquaculture
  - AlCare®, BioGrow®, BioPlus ®2B, NeoFerm BS10, LiquaLife®, etc.
- Most widely used and well studied strains in humans are:
  - ➢ *Bacillus Subtilis*
  - ➢ *Bacillus Licheniformis*
  - ➢ *Bacillus Coagulans*
  - ➢ *Bacillus Clausii*
  - ➢ *Bacillus Indicus HU36™*

Key Features of Bacterial Spores

- They form robust endospore and can withstand harsh temps, desiccation, low pH, gastric barriers, antibiotics, UV radiation, solvents, enzymes and even high pressures.
- They are found all over the environment (soil, vegetation, dust, rocks, aqua-environments, digestive systems of insects, marine life, mammals, etc.
- Spores Remain dormant for over 50 million years
- Found all over the ancient environment: ice-core studies
- They colonize very effectively in the Human GIT and have been found to colonize very effectively in the GIT of several different animals.
- Are found as part of the normal human commensal flora.
- LONG history of use in industries where efficacy is closely measured (pharma, agricultural)
- Extremely safe
- Its use as a probiotic is evolutionarily supported – true commensal organism.

Casula, G., & Cutting, S. (2002). Bacillus probiotics: Spore germination in the gastrointestinal tract. Applied & Environmental Microbiology 68(5):2344-2352.
Cutting, S. (n.d.). Bacillus probiotics-Mechanism of action and use. Protexin Healthcare.
Dong, T., Van, P., & Cutting, S. (2009). Bacillus probiotics. Nutra Foods 8(2):7-14.

FUNCTION OF SPORES IN THE HUMAN SYSTEM

Microbiota Balance

Spores produce at least 24 potent antibiotics that control bacterial over-growth in the GIT.

- ✓ Examples: Coagulin, Subtilisin, Amicoumacin, Surfactin, Iturins A, and Bacilysin

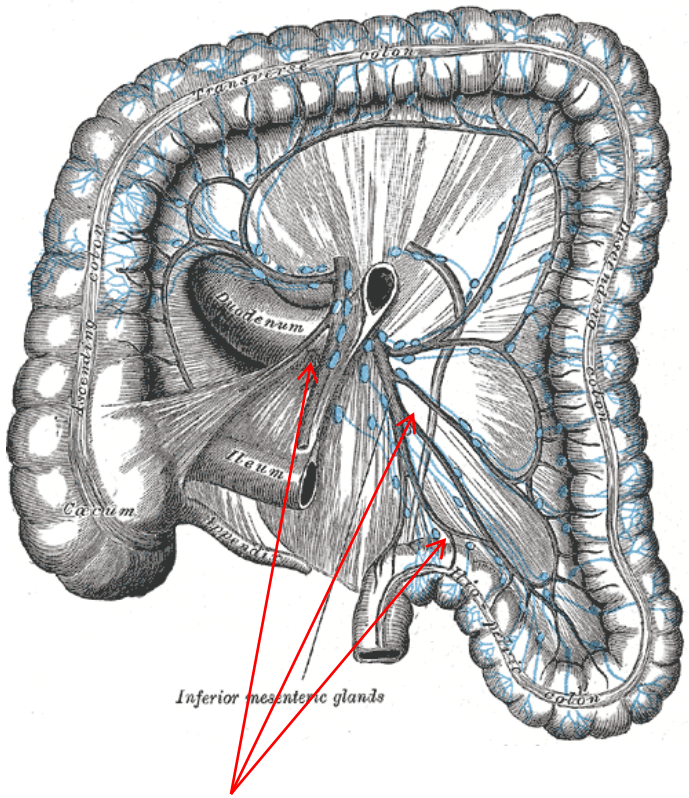
- **Spores conduct competitive exclusion** (CE) of pathogenic organisms to help maintain microbiota balance.
  - ✓ Accomplished by competition for space, nutrients and/or eliciting host response
  - ✓ Causes host elimination of invading species
- **Spores have the ability to increase the numbers of the important GIT commensals, such as *lactobacillus*.**

✓ Example: the capacity of *B. subtilis* to produce catalase and subtilisin has been reported to promote growth of *Lactobacillus* species.

✓ When co-cultured, *Bacillus subtilis* enhanced the growth and viability of *L. reuteri*, *L.*

FUNCTION OF SPORES IN THE HUMAN SYSTEM

Immunomodulation



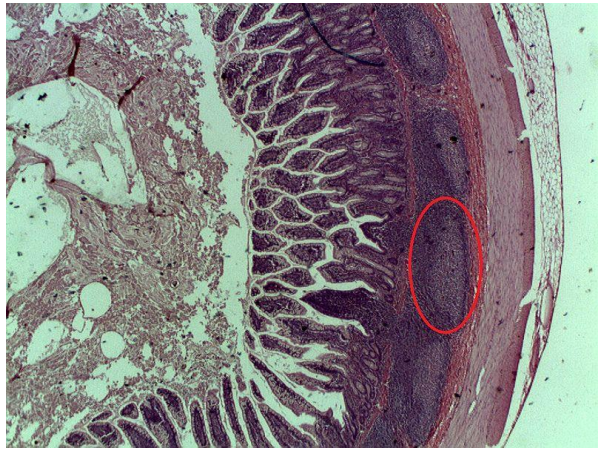
Mesenteric glands – sites of amplification

The intestines posses the largest amount of lymphoid tissue in the human body

GALT – Gut Associated Lymphoid Tissue

Peyer's Patches – found in the ileum of the small intestines. Maximum numbers found around age 15-24. Gut bacteria is a major player in development. Plays a major role in pathogen defense and self-recognition.

PPs favor a Th1 response via INF-g, TNF-a and IL-2



Immune sampling occurs in the lumen – an immune response is generated in the mucosa and then amplified in the mesenteric glands

FUNCTION OF SPORES IN THE HUMAN SYSTEM

ImmunoModulation: Bacillus Spores

- **Critical in the development of the GALT** itself by cooperation of *Bacteriodes Fragilis* sp. (largest population in the GIT)
- Produce potent activation and proliferation of lymphocytes in the Peyer's patch – **Promotes Th1 shift – Oral tolerance/mucosal tolerance of food proteins and non-pathogenic microbes. PP dysfunction linked to hypersensitivity, chronic Th2 activation and inflammatory conditions (celiac).**
- **Cause the production of important immune cytokines in mesenteric lymph nodes** (MLN) (IL-1a, IL-5, IL-6, IFN-g and TNF-a) and in the spleen (IFN-g and TNF-a)
- **Interacts with Toll-like receptors(TLR).** Vegetative cells of B. subtilis & other Bacillus sp. up-regulate expression of TLR2 and TLR4
    - Leads to amplified **innate immune response** via macrophages, monocytes, B-cells and Dendritic cells. Then push to **Adaptive Immunity.** Dendritic activation, important to create link between innate and adaptive immunity

FUNCTION OF SPORES IN THE HUMAN SYSTEM

Immune Modulation: Bacillus Spores

Autoimmune – **Bacillus has been shown to improve adaptive immunity via PP activation and TLR patter recognition – important self/non-self mechanism.**

- **Over active innate linked to autoimmune conditions – need for TLR expression to make adaptive switch.**
- Viral mimicry of self proteins invade our immune system (innoculum, vaccines antibodies, etc).
- Vulnerable from 0-7 years during immune development, loss of recognition of self partially responsible for low TLR activity during PP development.
- Damage to the MHC allows pathogens loose inside the cell to attack (centromeres, DNA, RNA, and other cell products, tissue, mucous membranes etc.)

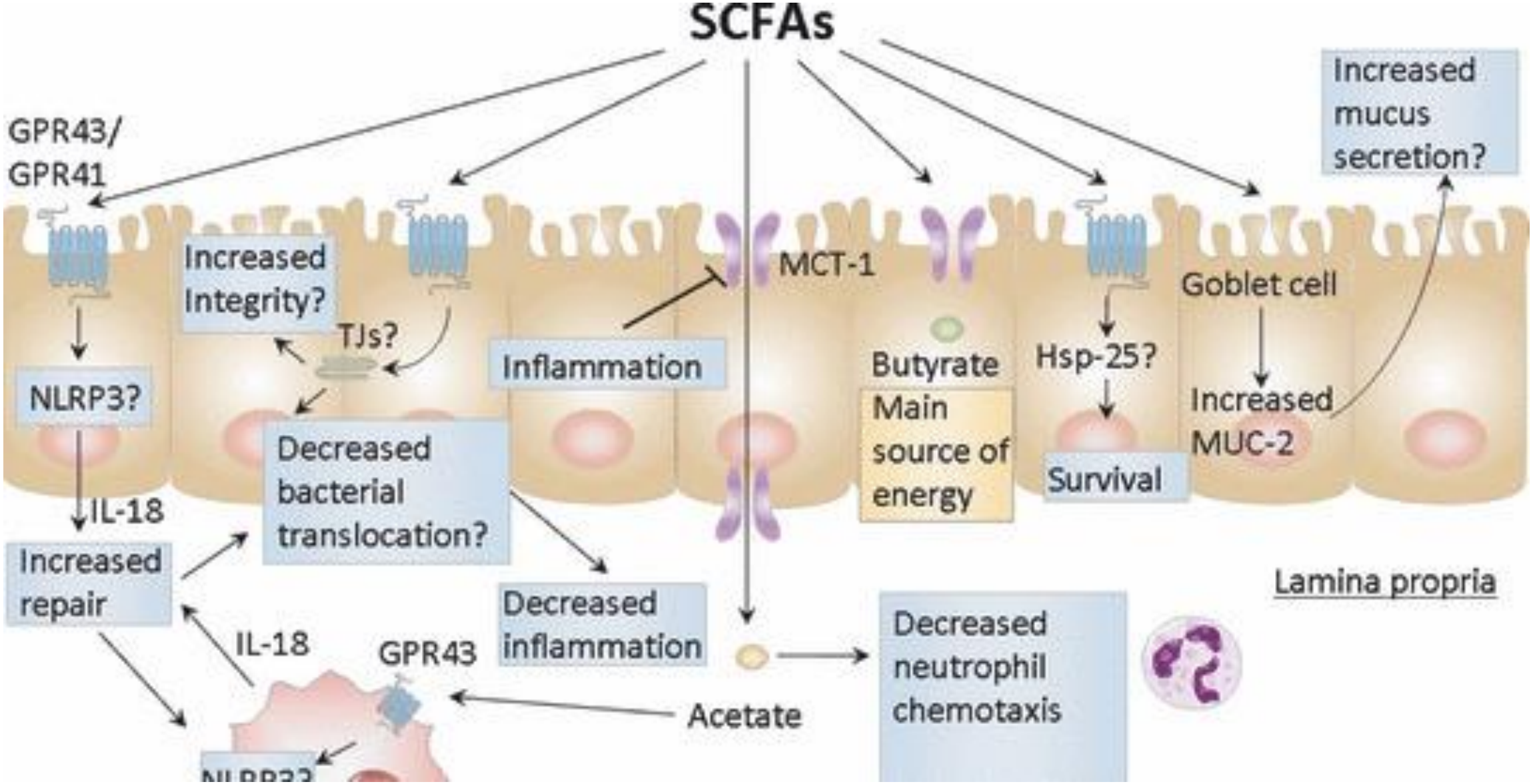
Allergies , Psoriasis, Eczema - direct correlation with GIT, especially helpful with babies born by C-Section

FUNCTION OF SPORES IN THE HUMAN SYSTEM

Food Intolerance and Th1/Th2 Shift :
Bacillus Spores

- Innate immunity (first line of defense) vs. adaptive
  - **Needs push to adaptive** to avoid chronic inflammatory state and damage to self tissue by NK cells and complement system.
  - Done via TLRs – stimulated by bacillus probiotics.
- Immune system of GIT is supposed to recognize food and create an adaptive response to decrease immune reactions – (function of Peyer's Patches M-Cells via antigen specific suppressor T-lymphocytes).
  - PP development and activation --- key to the function of this mechanism
- Latent viral (CMV, HHV-6, EBV, etc.) and bacterial infections maintain Th2 activation via IL10 mimic
  - Causes B-Cell class shift to IgE. This inhibits Th1 and allows for yeast growth.
  - Bacillus spores stimulate chemokine receptor CCR5 in GALT to turn on Th1. T-bet transcriptional factors are upregulated which promotes TH1 and suppresses GATA3 (the pro-TH2 factor). Secretion of IL-12, TNF-g and INF-g also push Th1 shift – all stimulated by *bacillus* spores.

Short Chain Fatty Acids and Immune Modulation



GUT PERMEABILITY – CHRONIC INFLAMMATION

Stress induces endotoxemia and increasing barrier permeability

Karin de Punder* and Leo Pruimboom
Frontiers in Immunology published: 15 May 2015

“Chronic non-communicable diseases (NCDs) are the leading causes of work absence, disability, and mortality worldwide. Most of these diseases are associated with low-grade inflammation.”

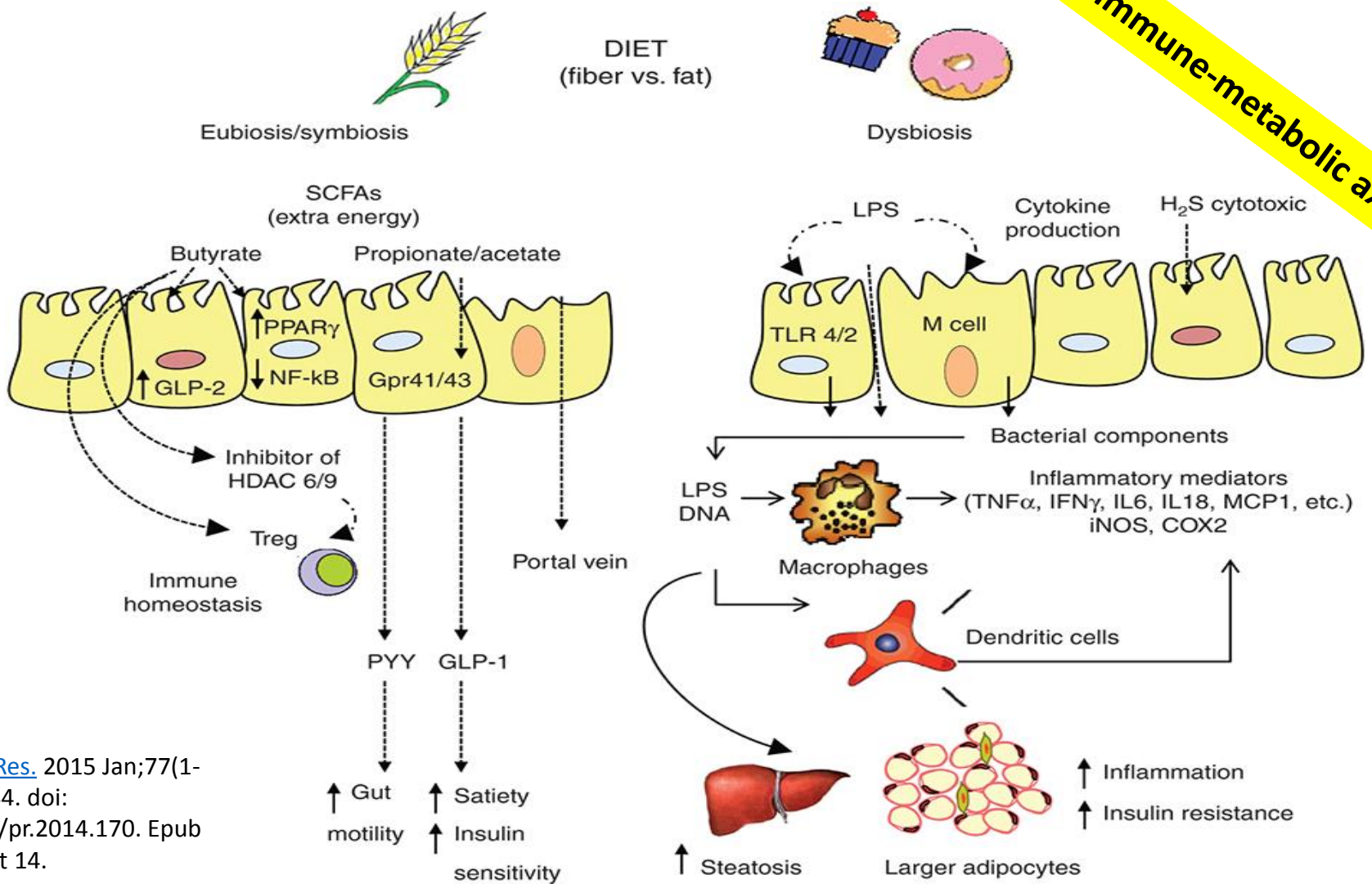
“In combination with modern life-style factors, the increase in **bacteria/bacterial toxin translocation** arising from a more **permeable intestinal** wall causes a low-grade inflammatory state. We support this hypothesis with numerous studies finding **associations with NCDs and markers of endotoxemia**, suggesting that this process plays a pivotal and perhaps even **a causal role** in the development of low-grade inflammation and its related diseases.”

GROUND ZERO OF MOST HEALTH DISORDERS

LEAKY GUT – THIS JUST MAY BE THE SOLUTION

- Post-prandial Endotoxemia

microbiota - immune-metabolic axis



Pediatr Res. 2015 Jan;77(1-2):236-44. doi: 10.1038/pr.2014.170. Epub 2014 Oct 14.

LEAKY GUT – THIS JUST MAY BE THE SOLUTION

J Interferon Cytokine Res. 2016 Feb;36(2):**Effects of Bacillus subtilis on Epithelial Tight Junctions of Mice with Inflammatory Bowel Disease.**
Gong Y[1], Li H[1], Li Y[1].

"B. subtilis intake upregulated expression of TJ proteins(claudin-1, occludin, JAM-A, and ZO-1), for improved barrier function, and downregulated cytokine expression (IL-6, IL-17, IL-23, and TNF-α) to reduce intestinal epithelial damage."

Comp Biochem Physiol A Mol Integr Physiol. 2002 Sep;133(1):95-104.
Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto.
Samanya M[1], Yamauchi KE.

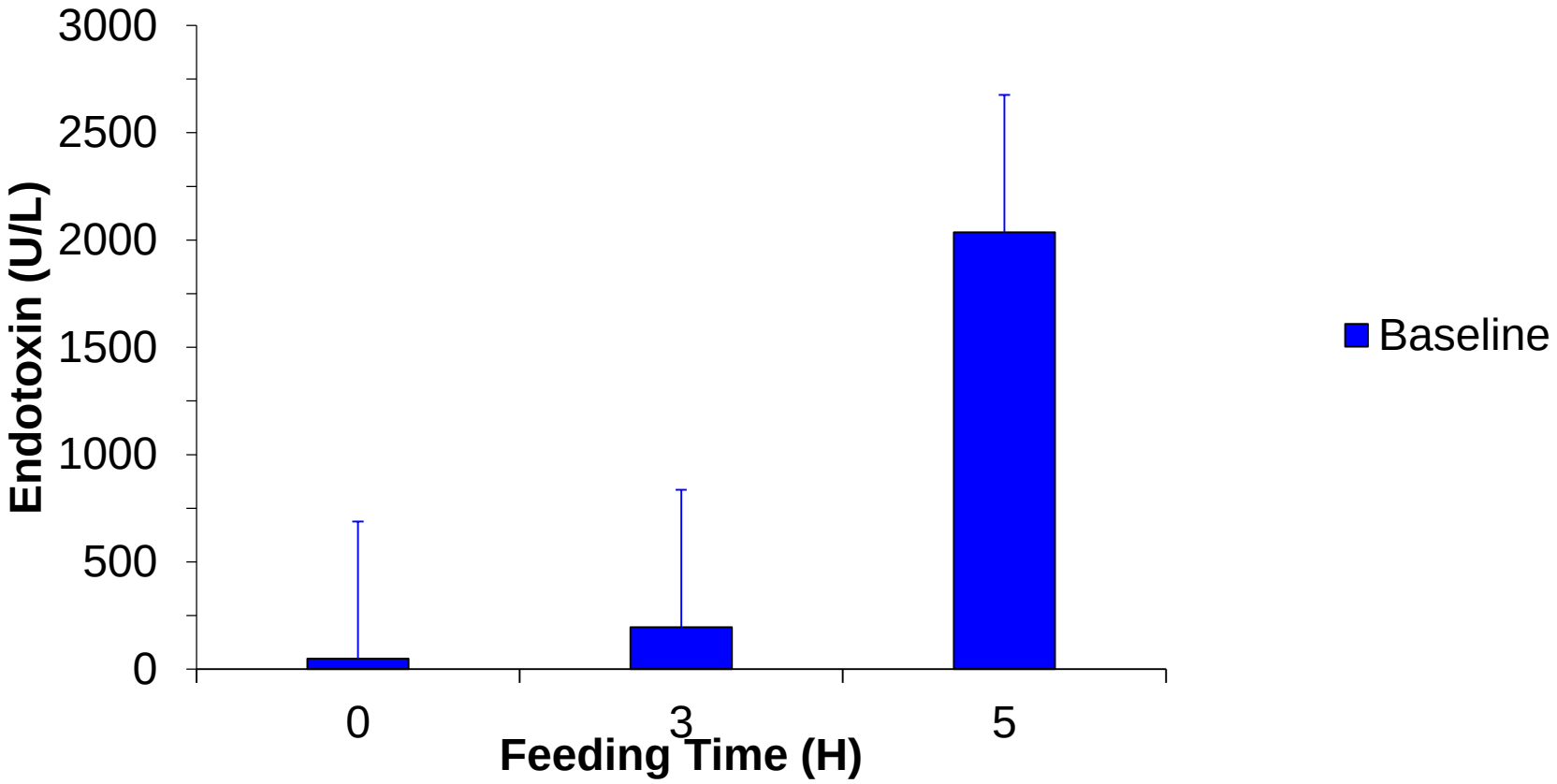
"These birds had a tendency to display greater growth performance and intestinal histologies, such as villus height, cell area and cell mitosis, than the controls. "

***Bacillus subtilis* Protects Porcine Intestinal Barrier from Deoxynivalenol via Improved Zonula Occludens-1 Expression**
Min Jeong Gua, Sun Kwang Songa, Sung Moo Park

"B. subtilis may have potential to enhance epithelial barrier function and to prevent the cells from DON-induced barrier dysfunction."

The effect of 30-days of probiotic supplementation on post-prandial responses to a high-fat meal: Pilot Study

Principal Investigator: Brian K. McFarlin, PhD, FACSM, FTOS
University of North Texas



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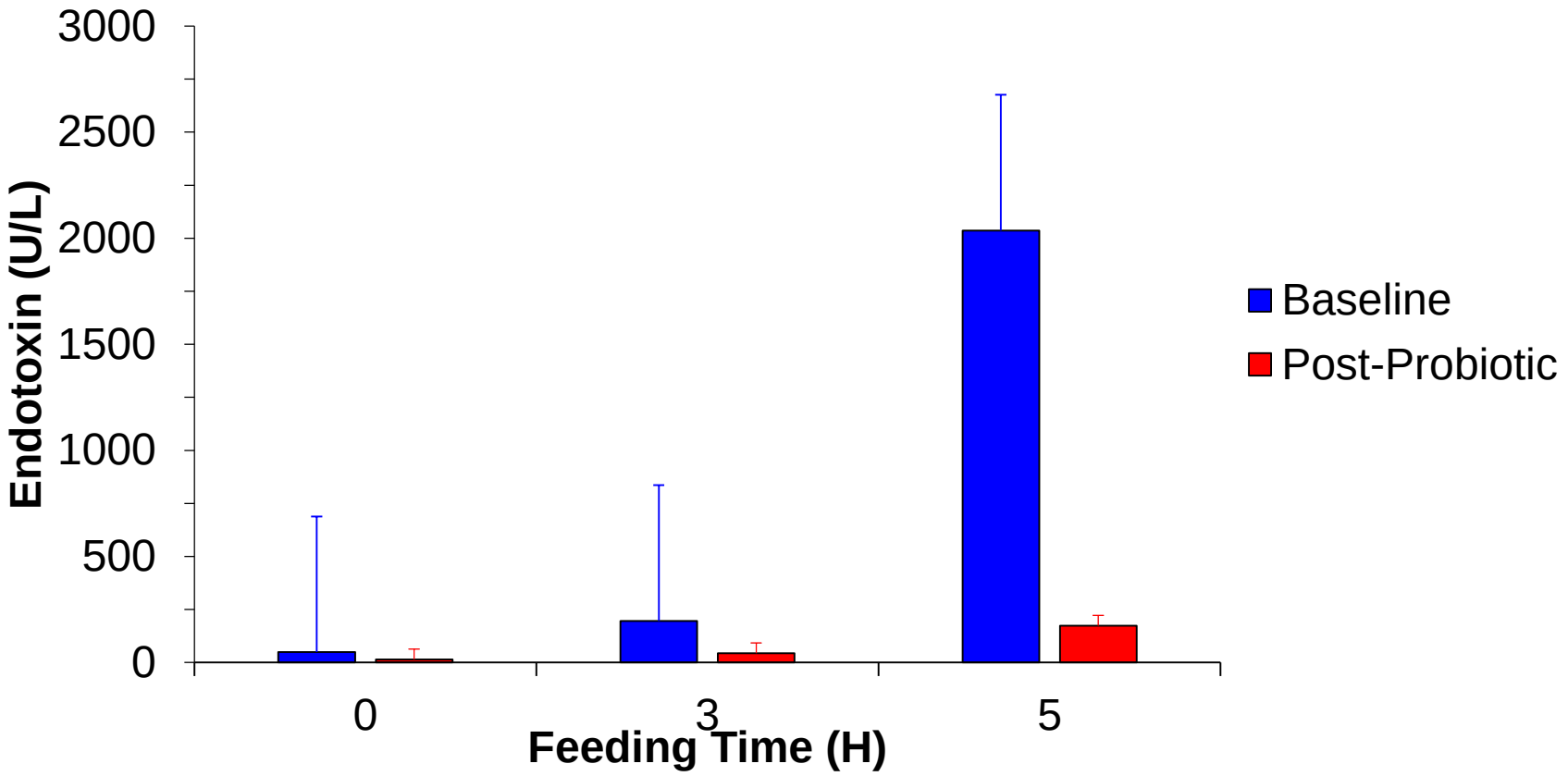
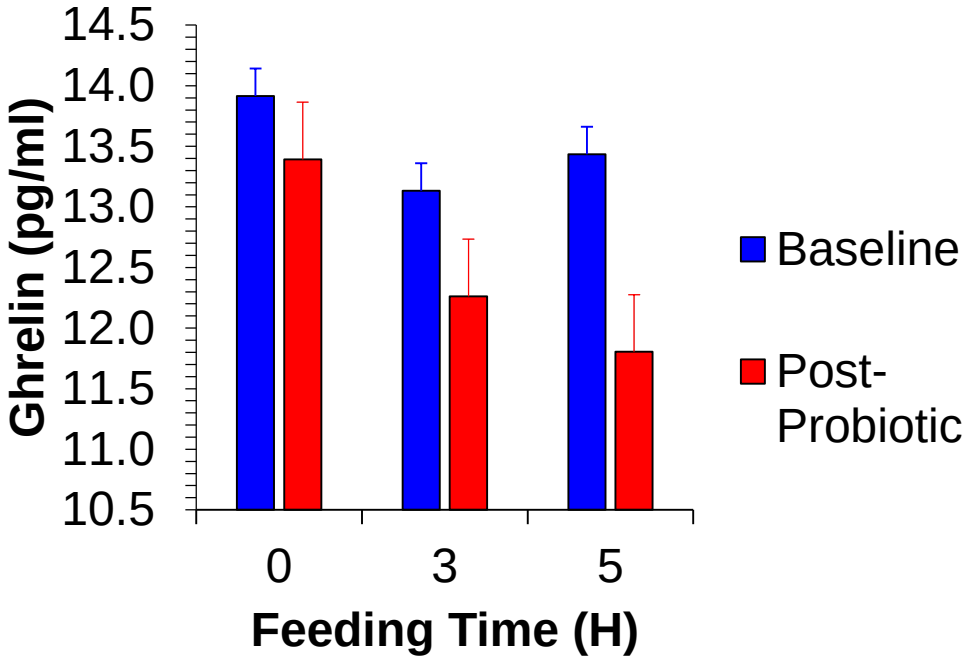
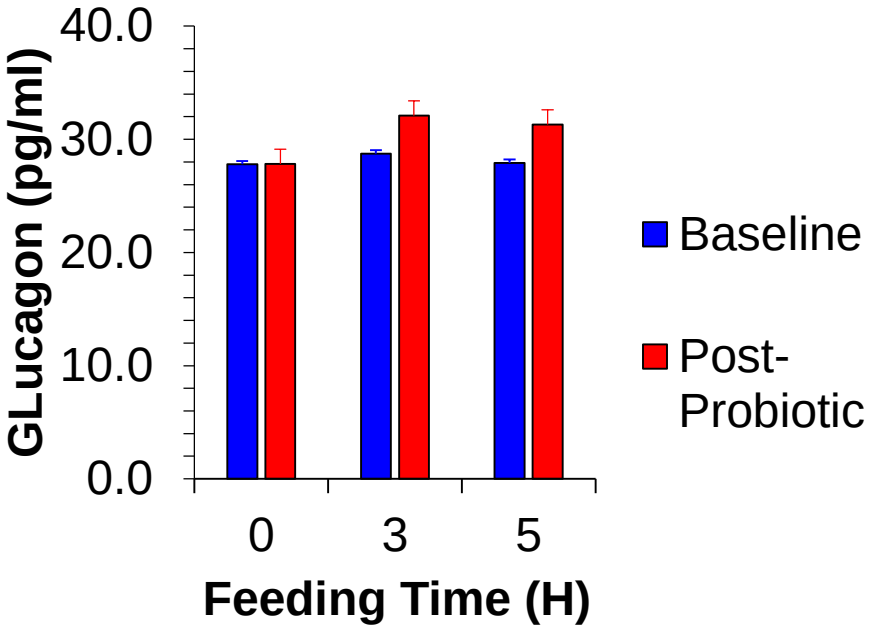
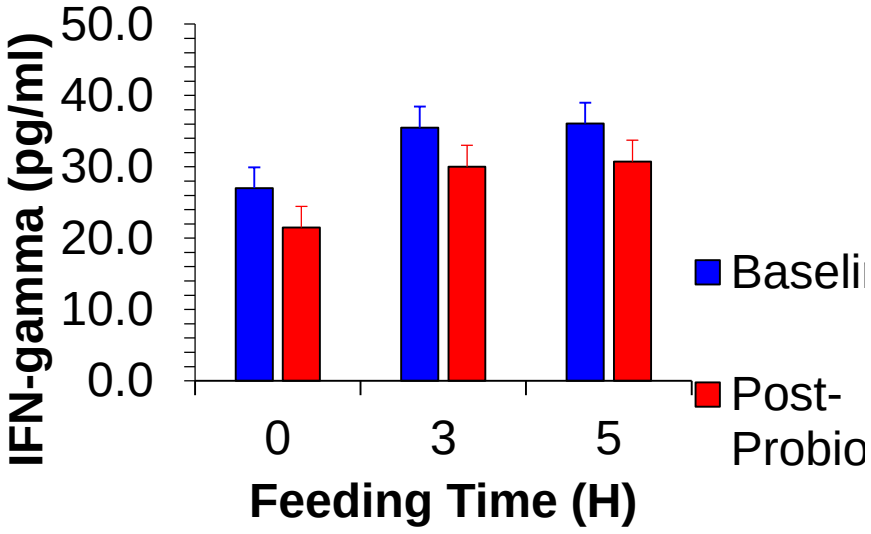
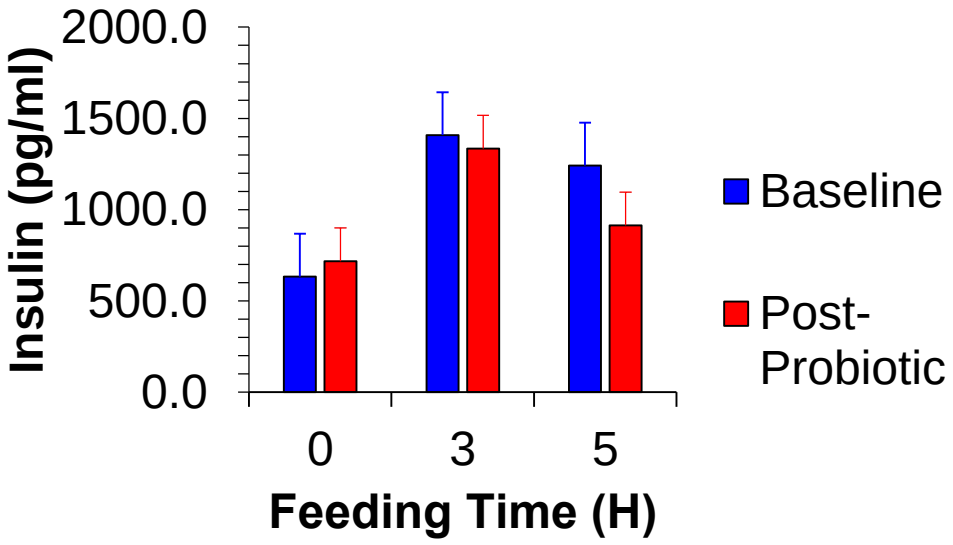



Ghrelin (pg/ml)
14.5
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
0
3
5
Feeding Time (H)
Baseline
Post-
Probiotic


GLucagon (pg/ml)
40.0
30.0
20.0
10.0
0.0
0
3
5
Feeding Time (H)
Baseline
Post-
Probiotic


IFN-gamma (pg/ml)
50.0
40.0
30.0
20.0
10.0
0.0
0
3
5
Feeding Time (H)
Baseli
Post-
Probio


Insulin (pg/ml)
2000.0
1500.0
1000.0
500.0
0.0
0
3
5
Feeding Time (H)
Baseline
Post-
Probiotic

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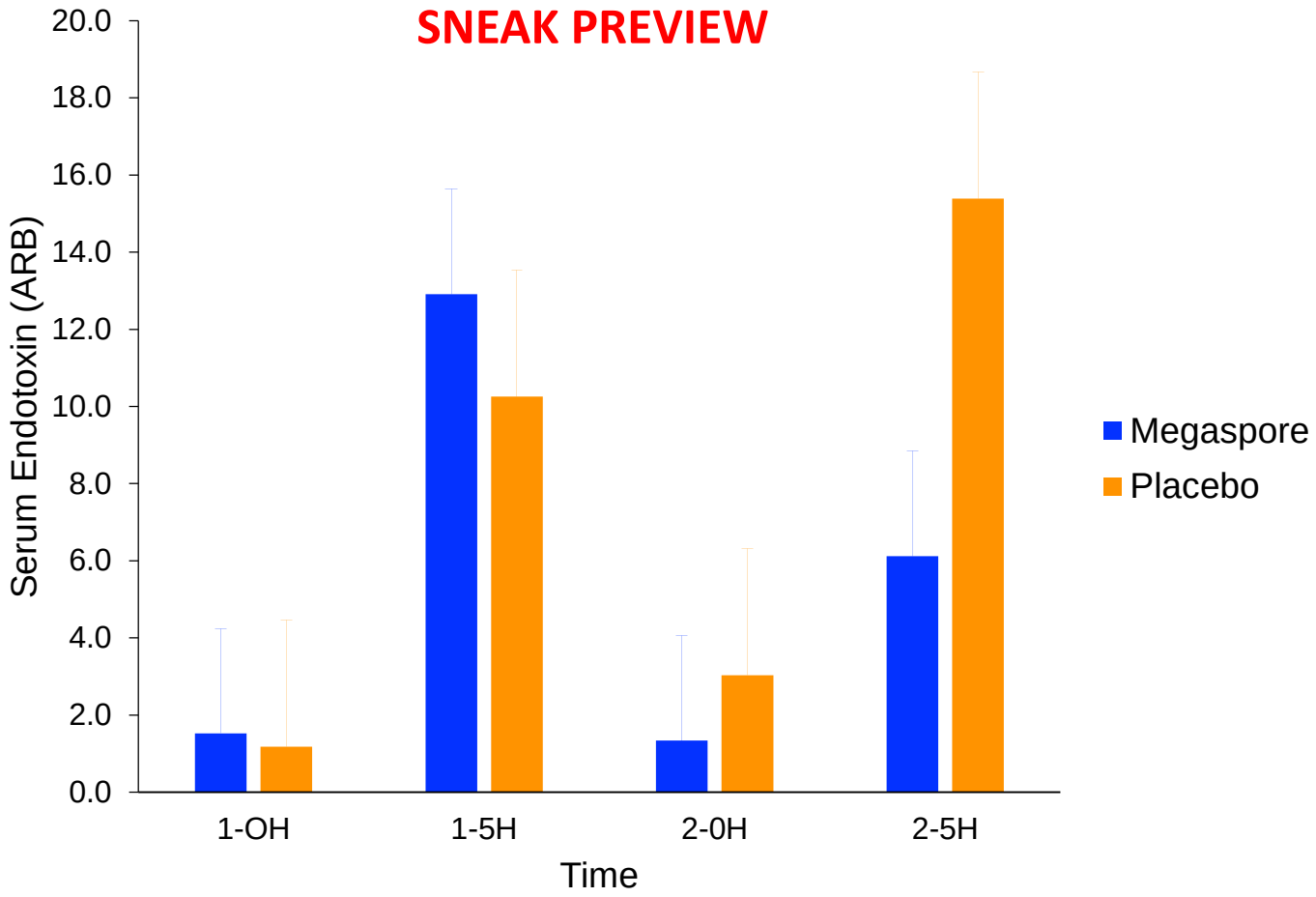

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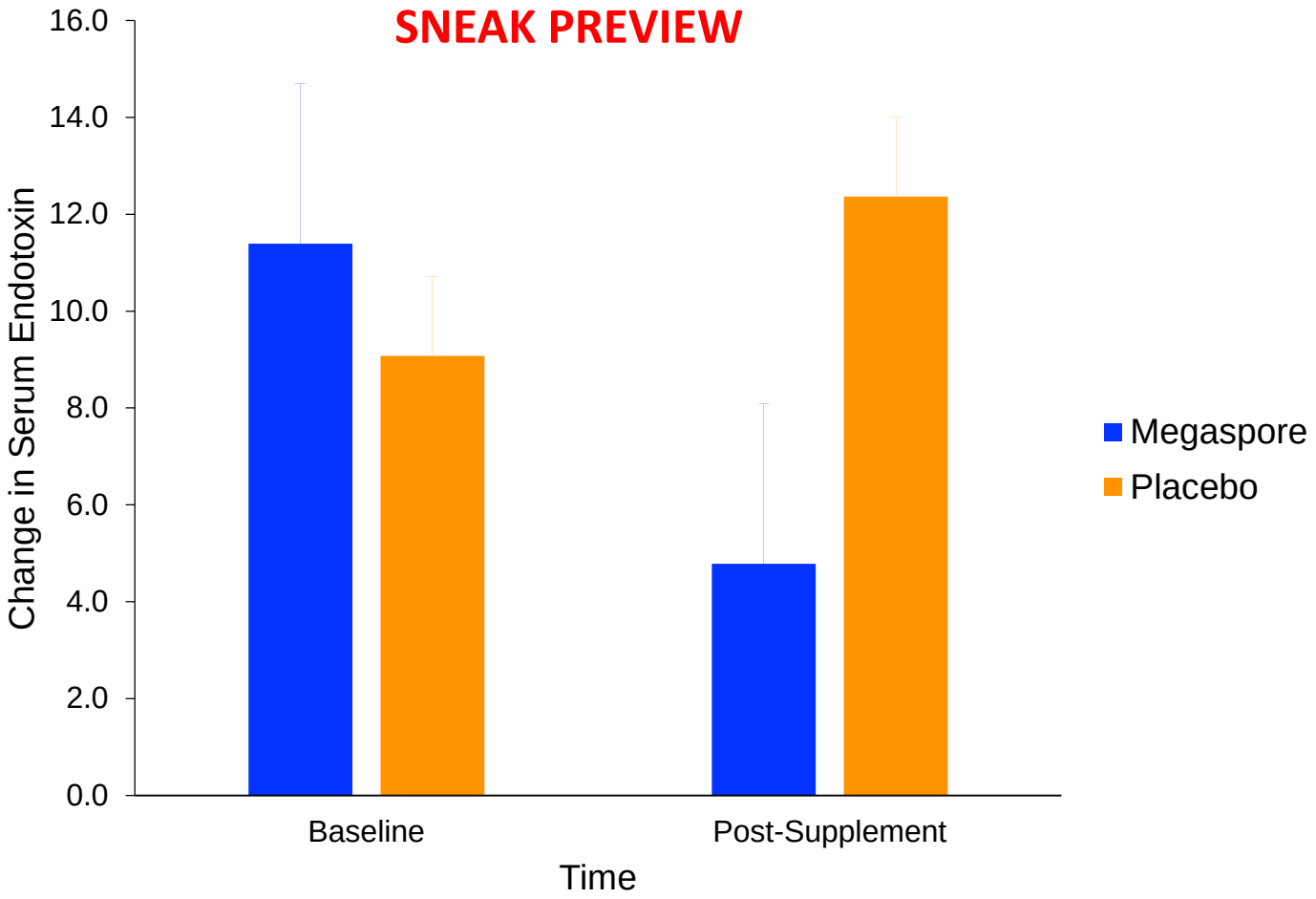

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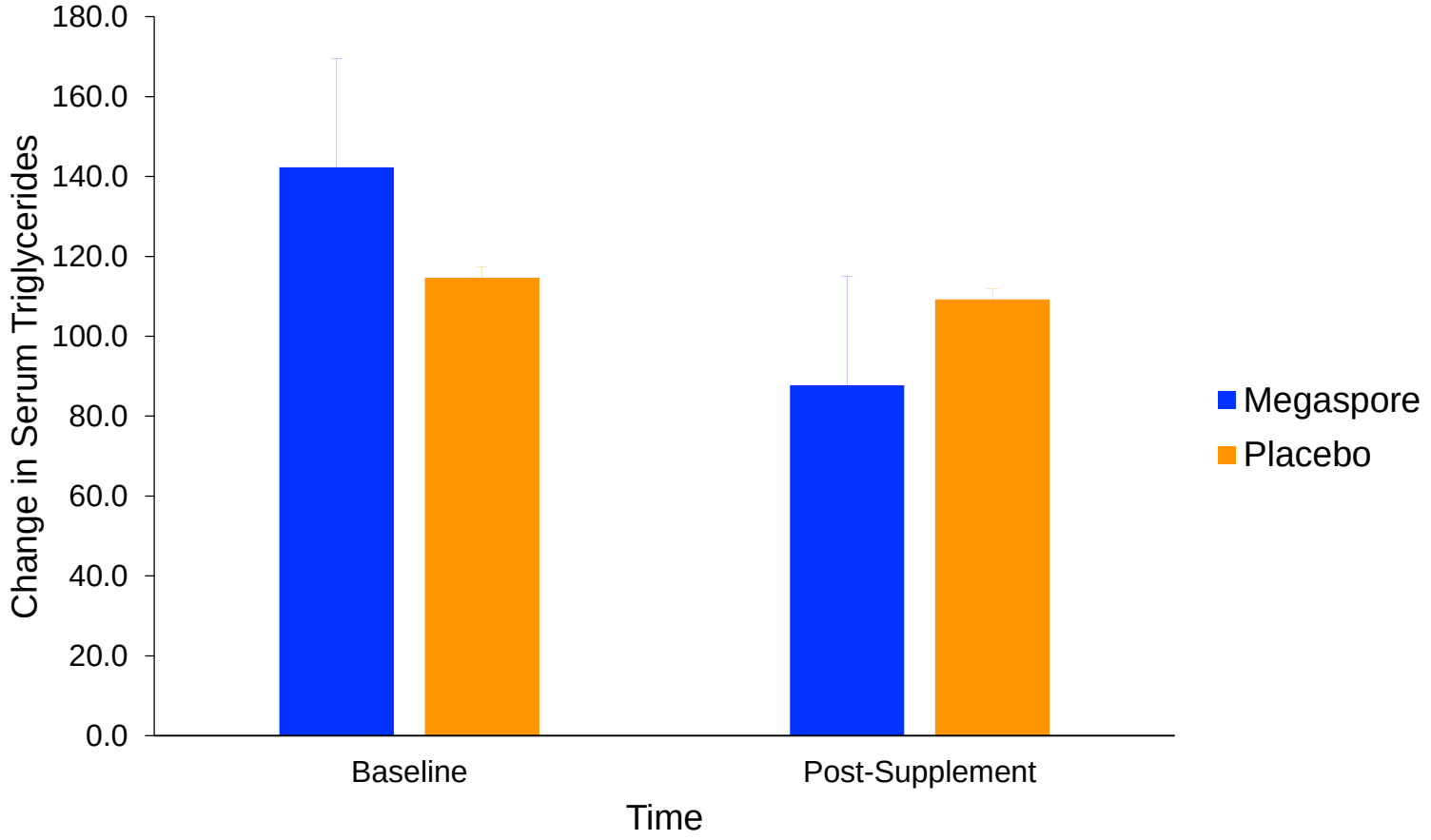
SNEAK PREVIEW



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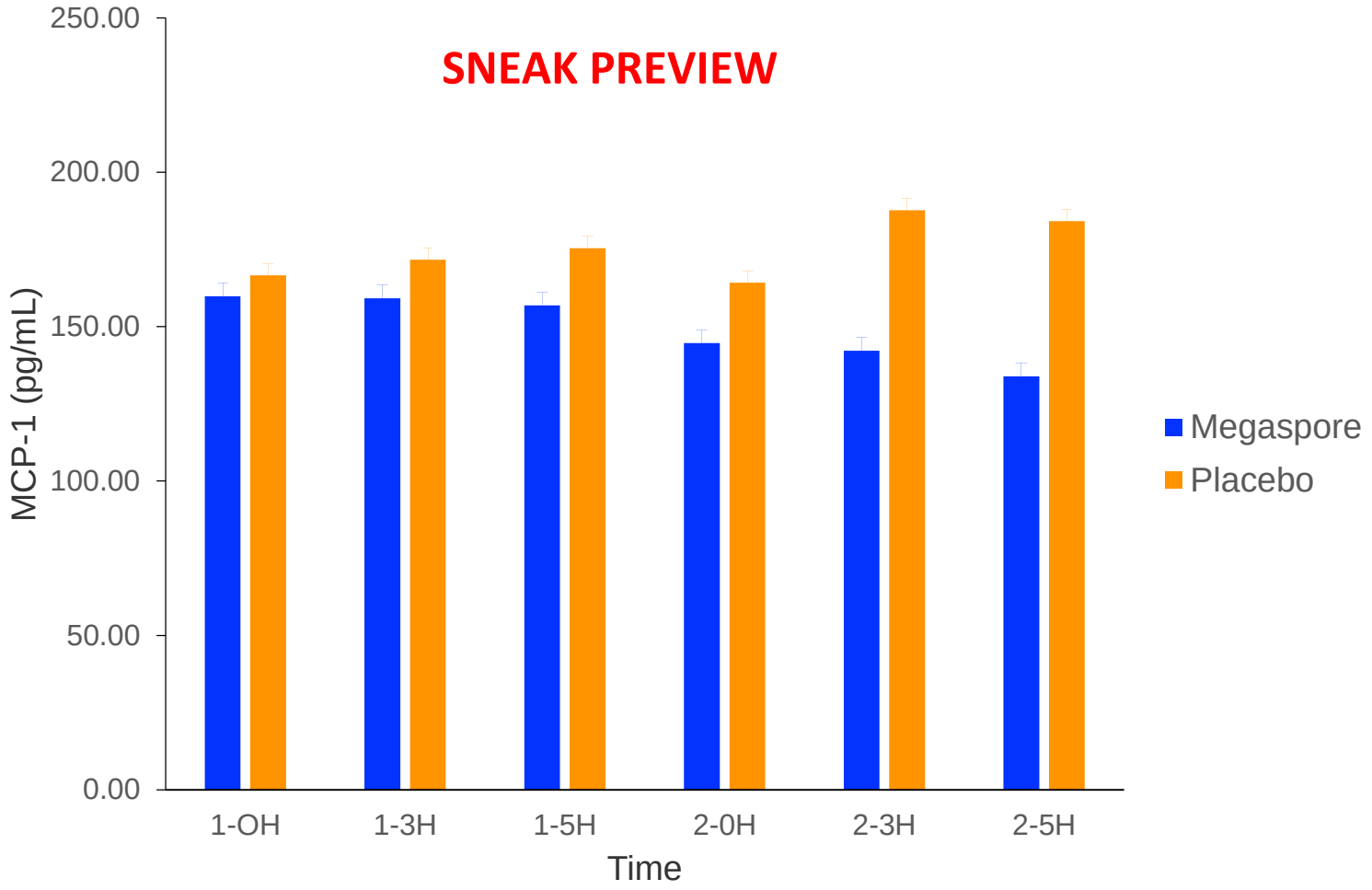
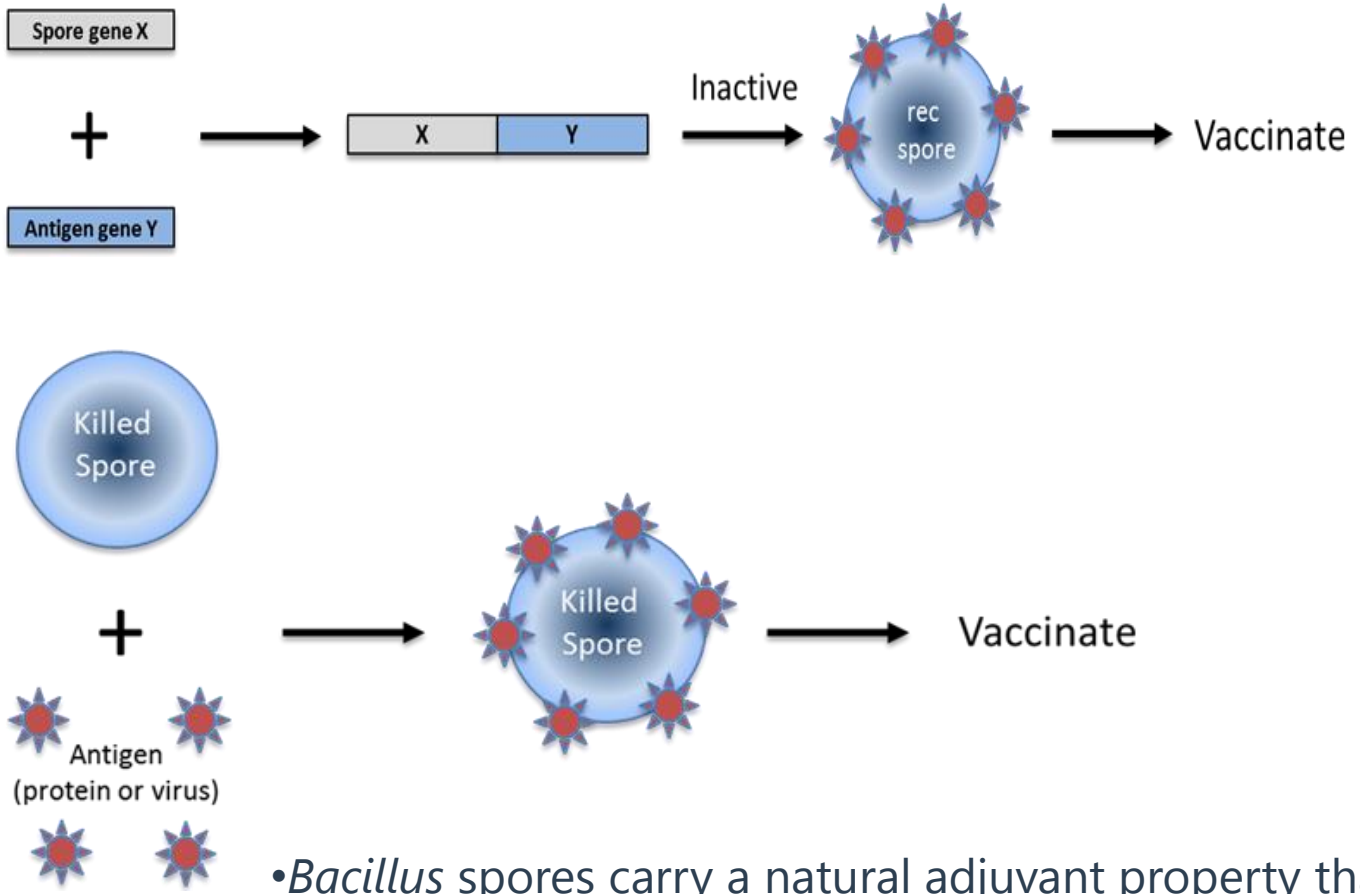


SporeGen

SporeVax®



Condition	Delivery Route	Protection
Tetanus	Oral	√
	Nasal	√
Clostridium perfringens	Oral	√
	Nasal	√
Anthrax	Nasal	√
Influenza	Nasal	√
Clostridium difficile	Oral	√
	Sub-lingual	√

•*Bacillus* spores carry a natural adjuvant property that enhances the body's natural immune responses. Typically, they produce balanced Th1 and Th2 responses as well as stimulating the innate immune system, an important primer for long-term immunity.