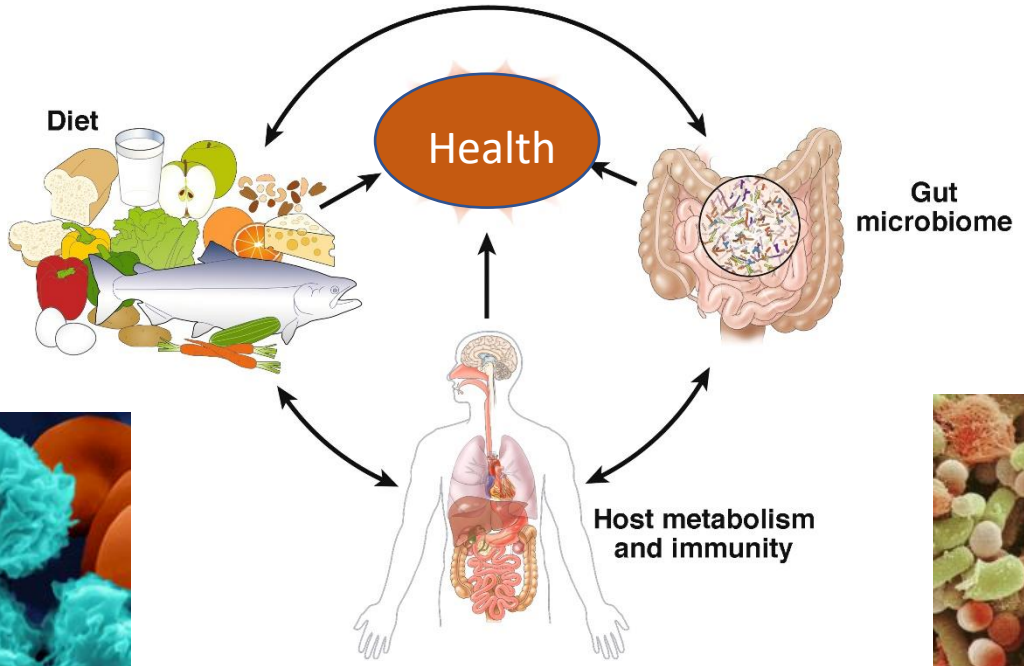
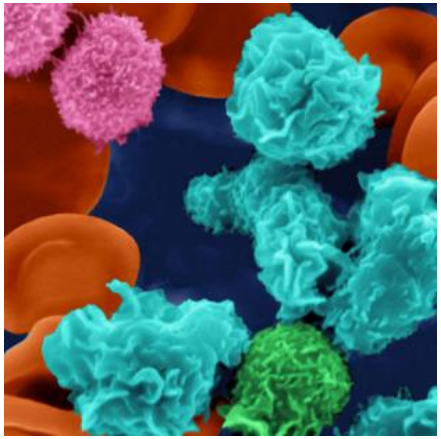
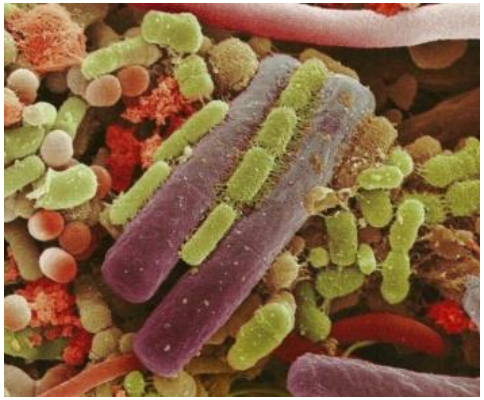
The role of the microbiome and the gut barrier in the ruminant mucosal immune system







Huub F.J. Savelkoul

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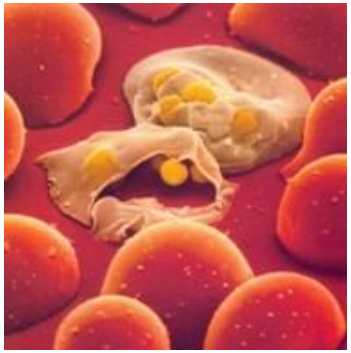
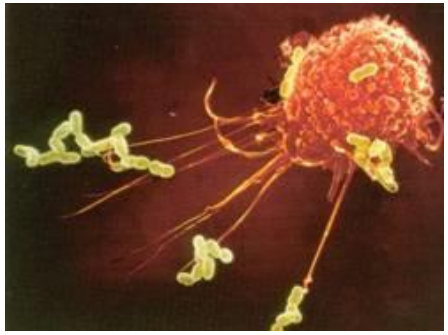
huub.savelkoul@wur.nl

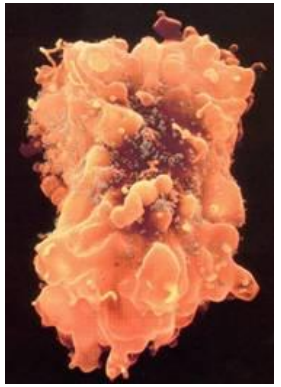
Functions of the immune system

The total area for protection is 2 m^2 skin, 80 m^2 lung, and 350 m^2 gut tissue.

	Human	Cow
skin	2 m^2	6-8 m^2
lung	80-100 m^2 6 l	150-180 m^2 12-13 l
gut	7.5 m 100-300 m^2	46 m (33-63) >2000 m^2





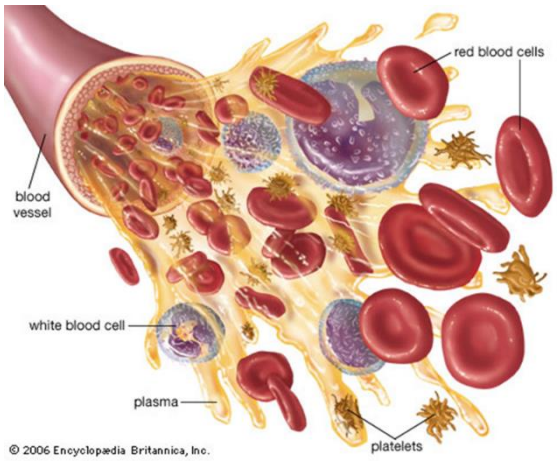


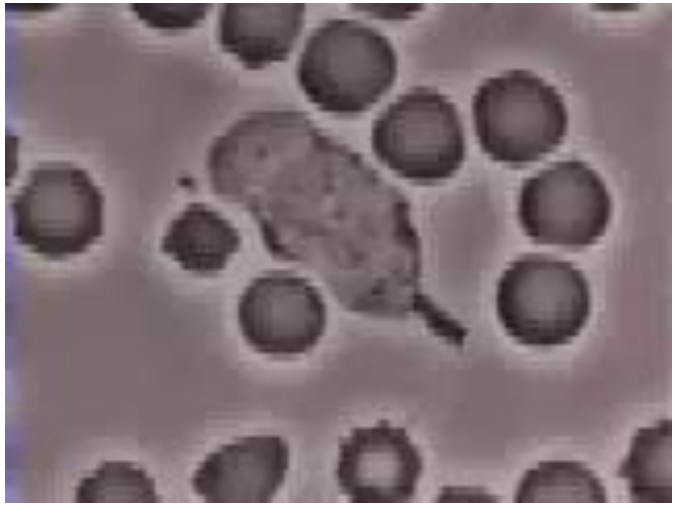
Infection =

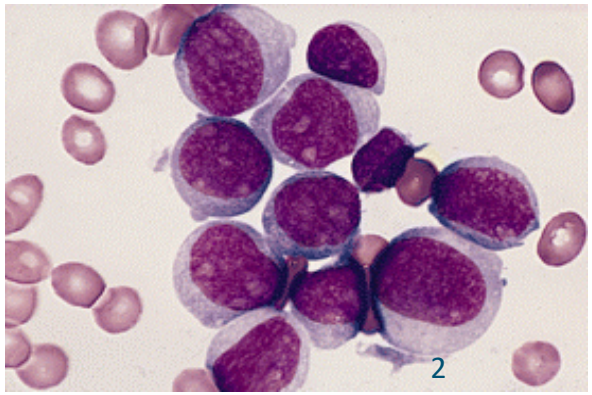
Non-self

Tumour =

Modified-self

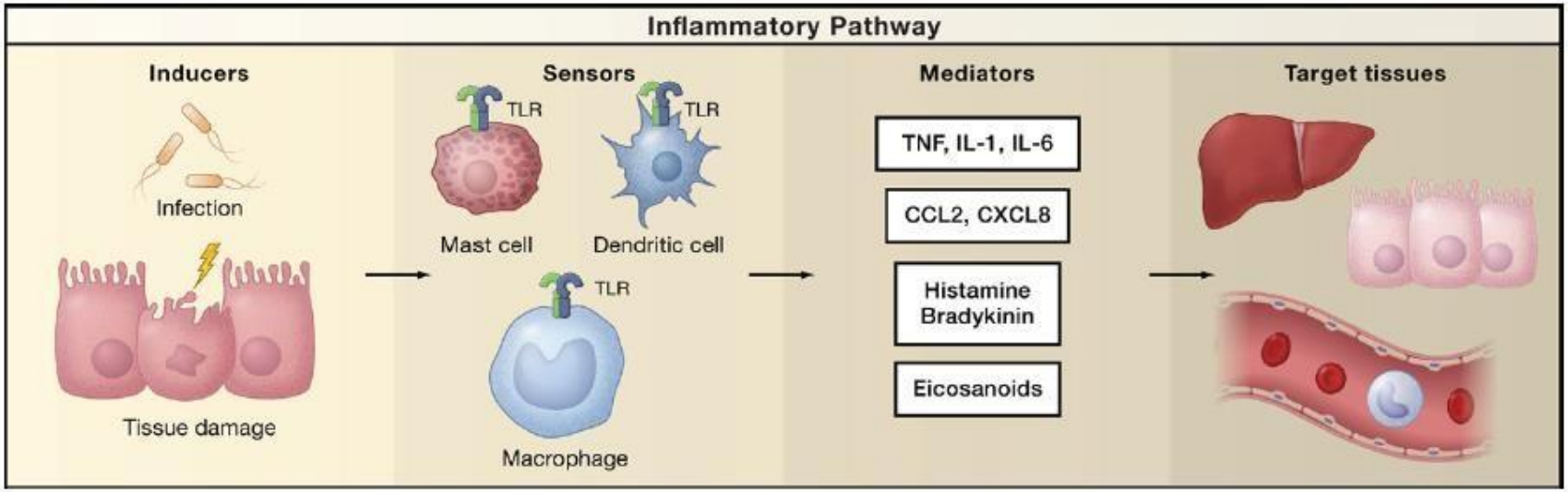






Acute and chronic inflammation

Acute inflammation is the immediate defensive reaction to infections or injuries



Vasodilation
Immune cell recruitment
RONS and interleukins

Resolution and health in 2 weeks or less

If the inflammatory inducer is not eliminated

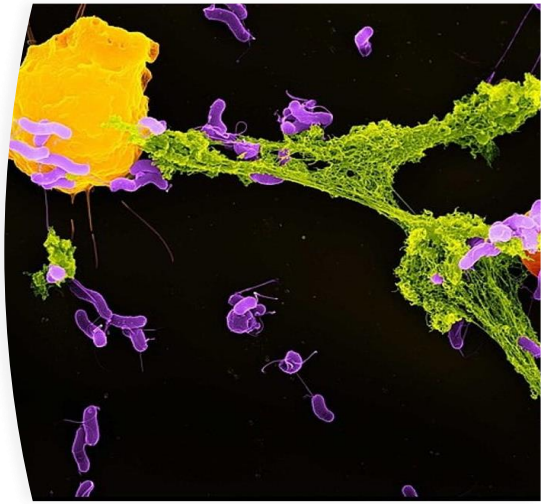
by the acute phase response

Immune cell infiltration
Increased production of inflammatory mediators

Chronic inflammation

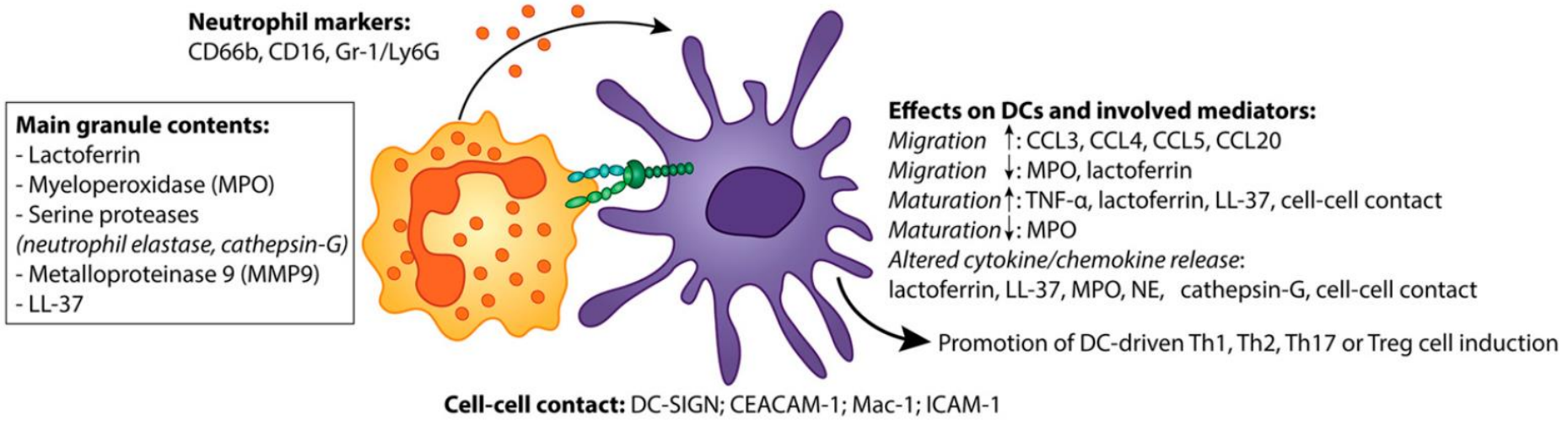
Medhhzitov, Cell 2010

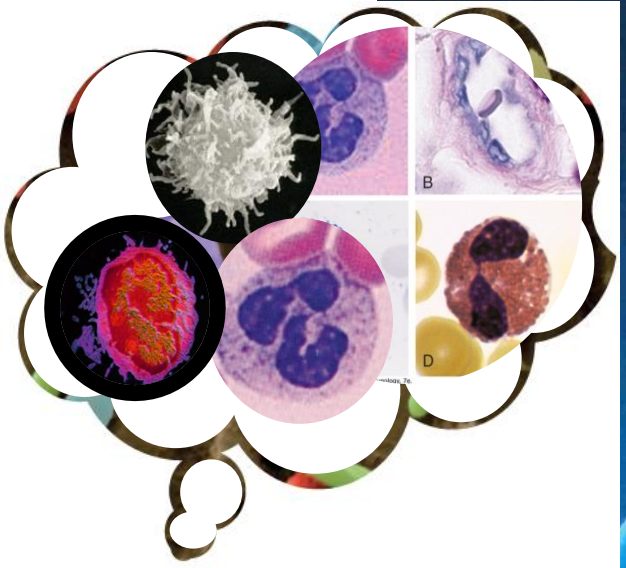
Neutrophils



- First responders of the innate immune system
- Can form NETs to trap and kill pathogens
  - Sticky, extracellular webs of nuclear DNA decorated with anti-microbial peptides
- Priming
  - IFNs
- Regulation innate but also adaptive immune responses
  - Interaction with DC

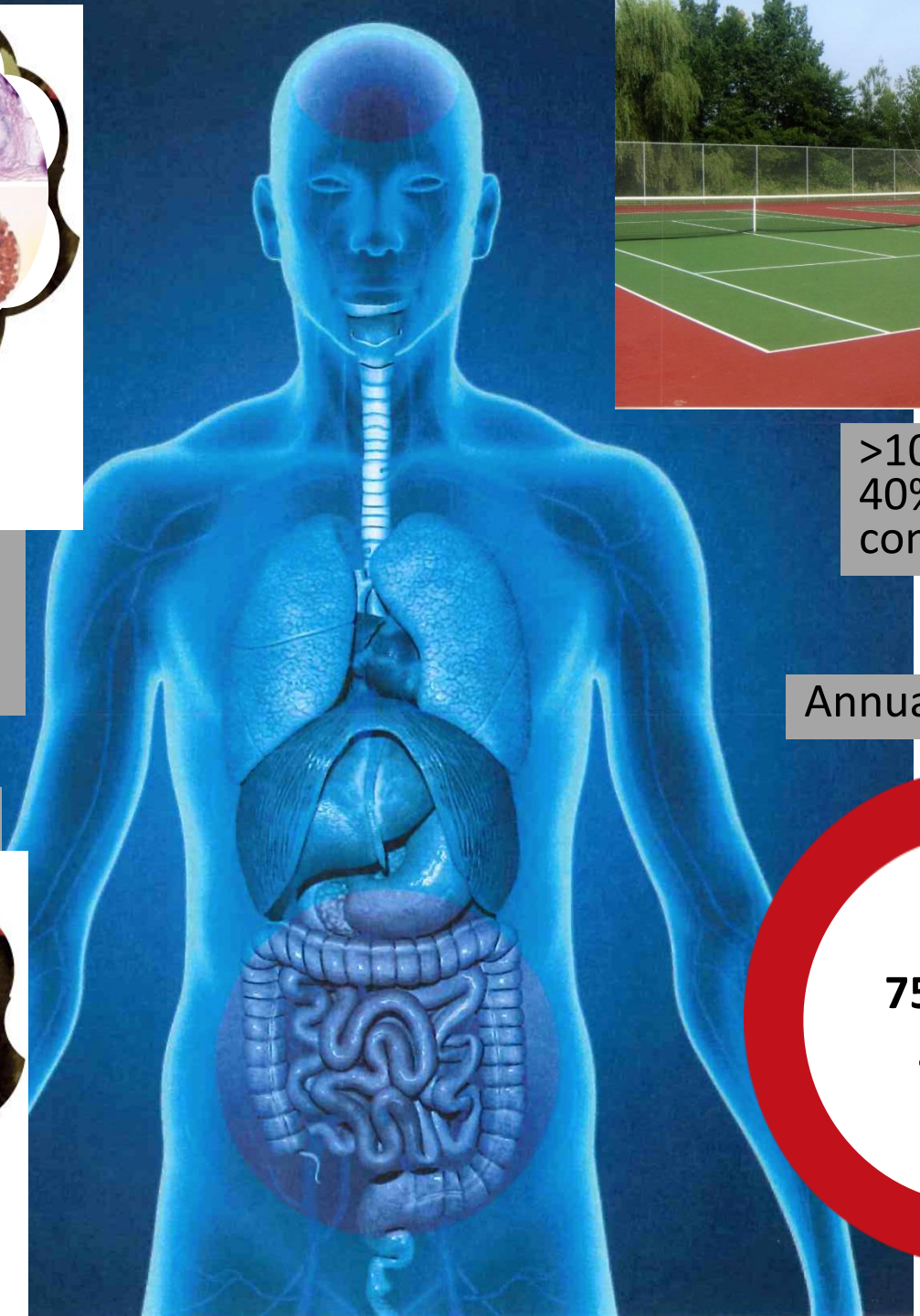
Breedveld et al. J Leuk Biol 103:1003; 2017



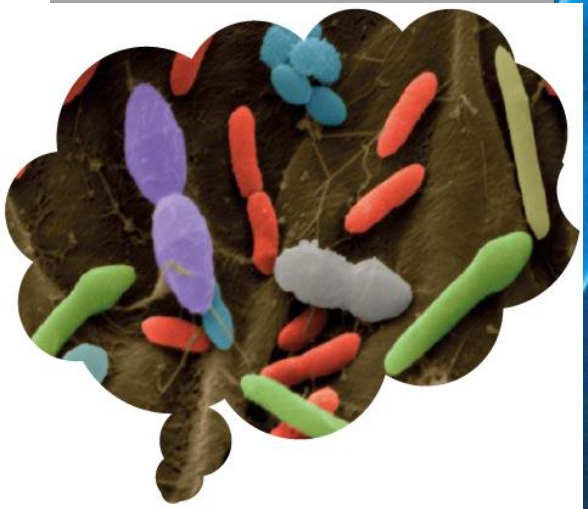


1.5 kg immune system
$50x10^9$ immune cells
70% in gut

>100 m^2 surface
40% energy consumption



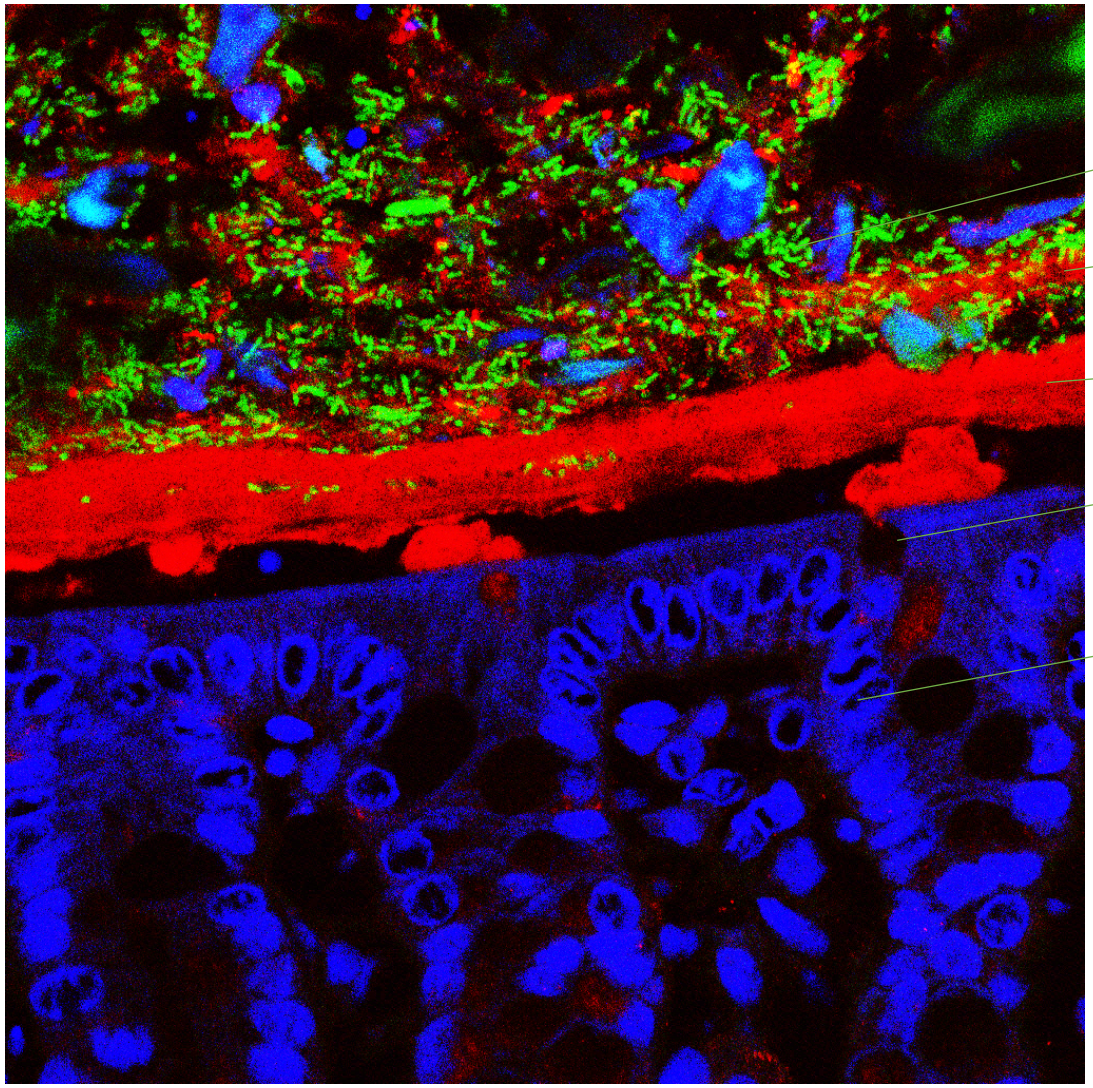
1.5 kg gut microbiota



Annual food intake

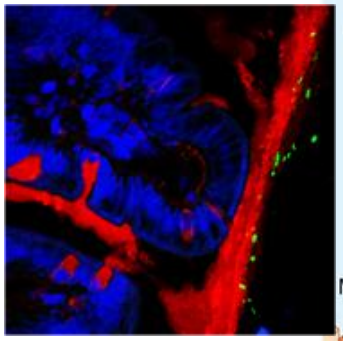
750 kg/yr
50 ton

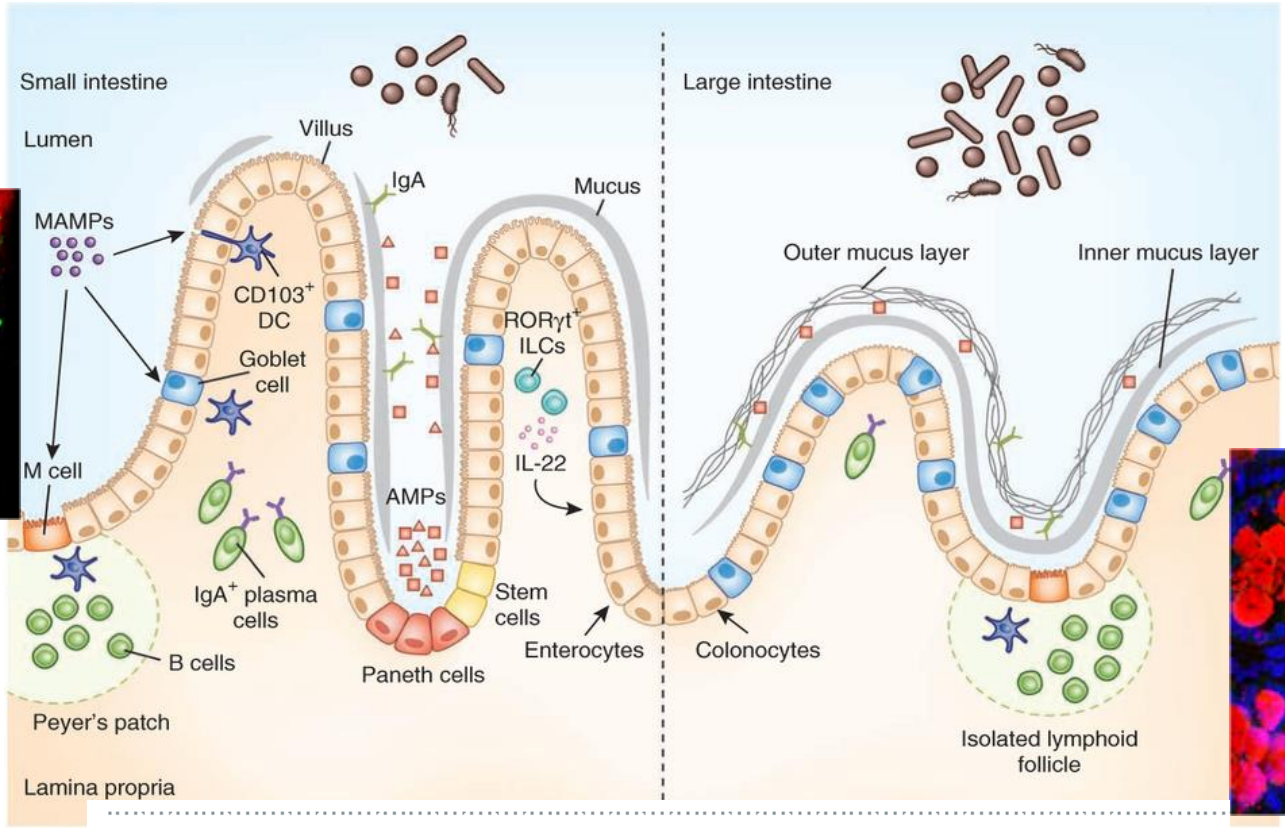
The epithelial barrier in mouse colon stained for nuclei (blue) mucus (red) and bacteria (green)

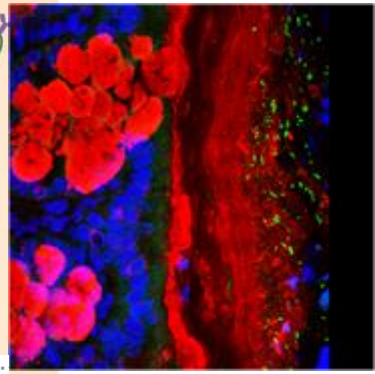


Sovran, van Beek, 2015

Host – microbe interaction in the gut





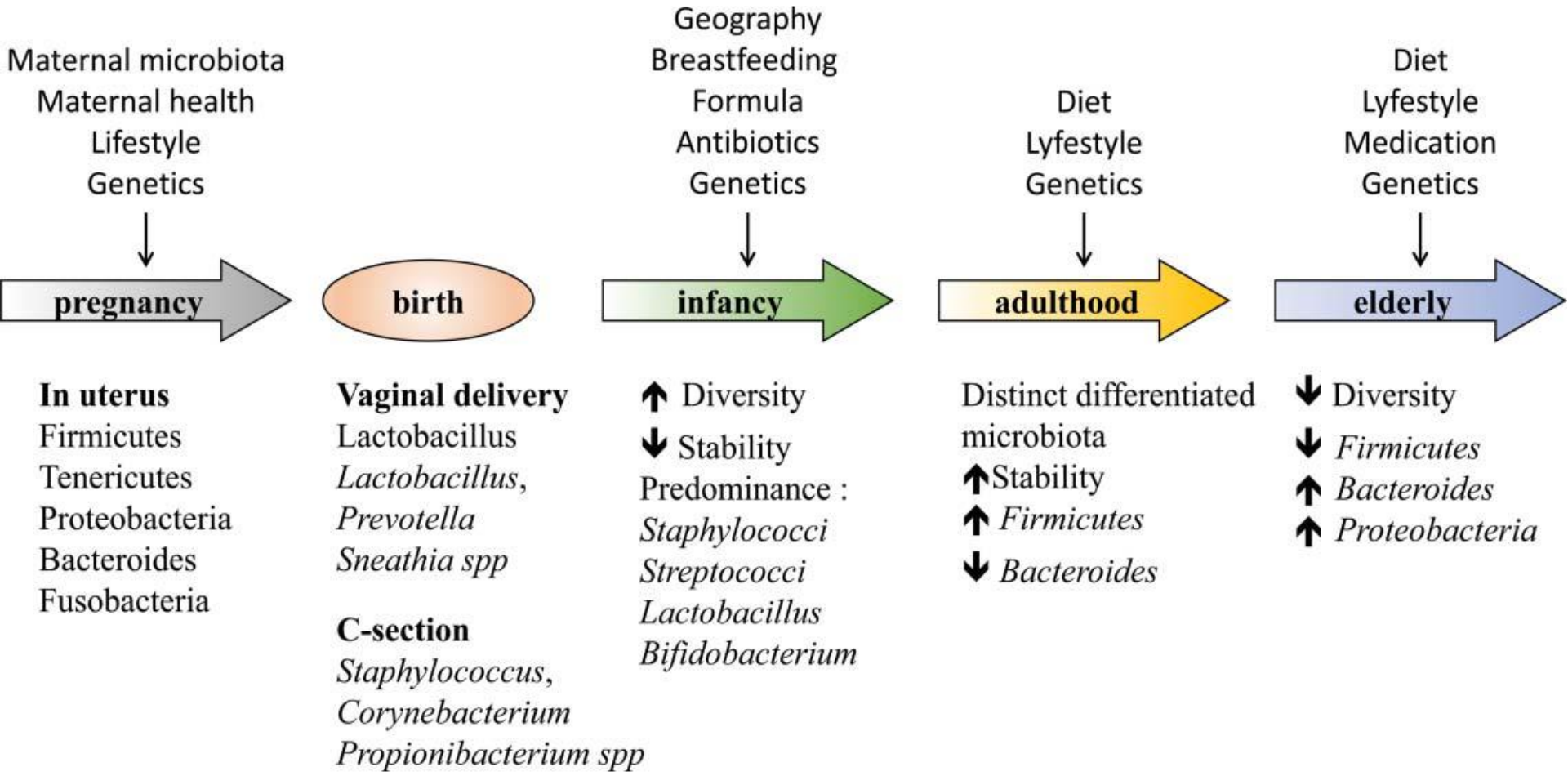


Sovran et al., Sci Report (2019) 9:1437

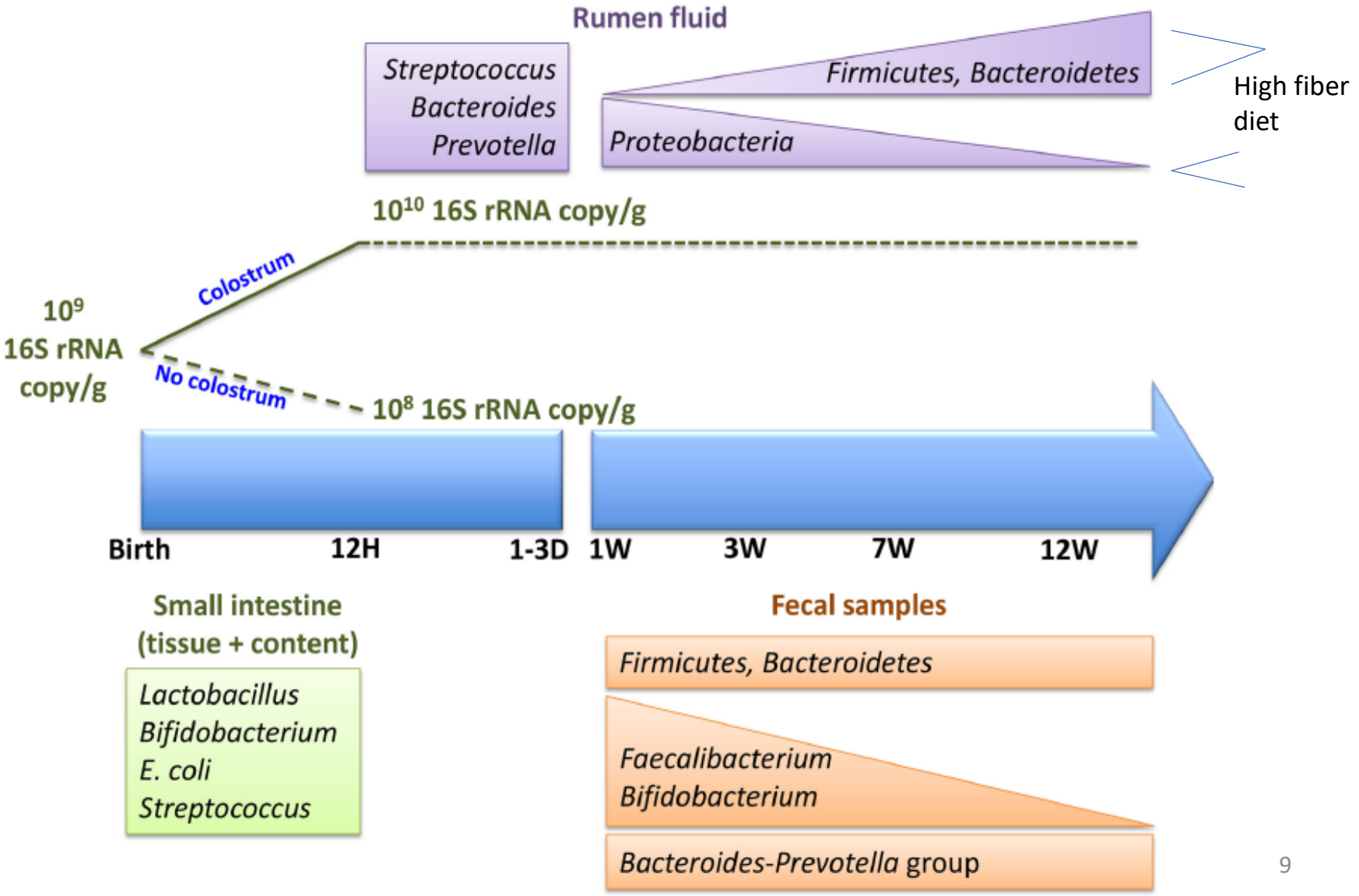
Age-associated Impairment of the Mucus Barrier Function is Associated with Profound Changes in Microbiota and Immunity

Bruno Sovran[1,3], Floor Hugenholtz[5], Marlies Elderman[1,4], Adriaan A. Van Beek[1,3], Katrine Graversen[2], Myrte Huijskes[2], Mark V. Boekschoten[1,6], Huub F. J. Savelkoul[1,3], Paul De Vos[1,4], Jan Dekker[1,2] & Jerry M. Wells[1,2]

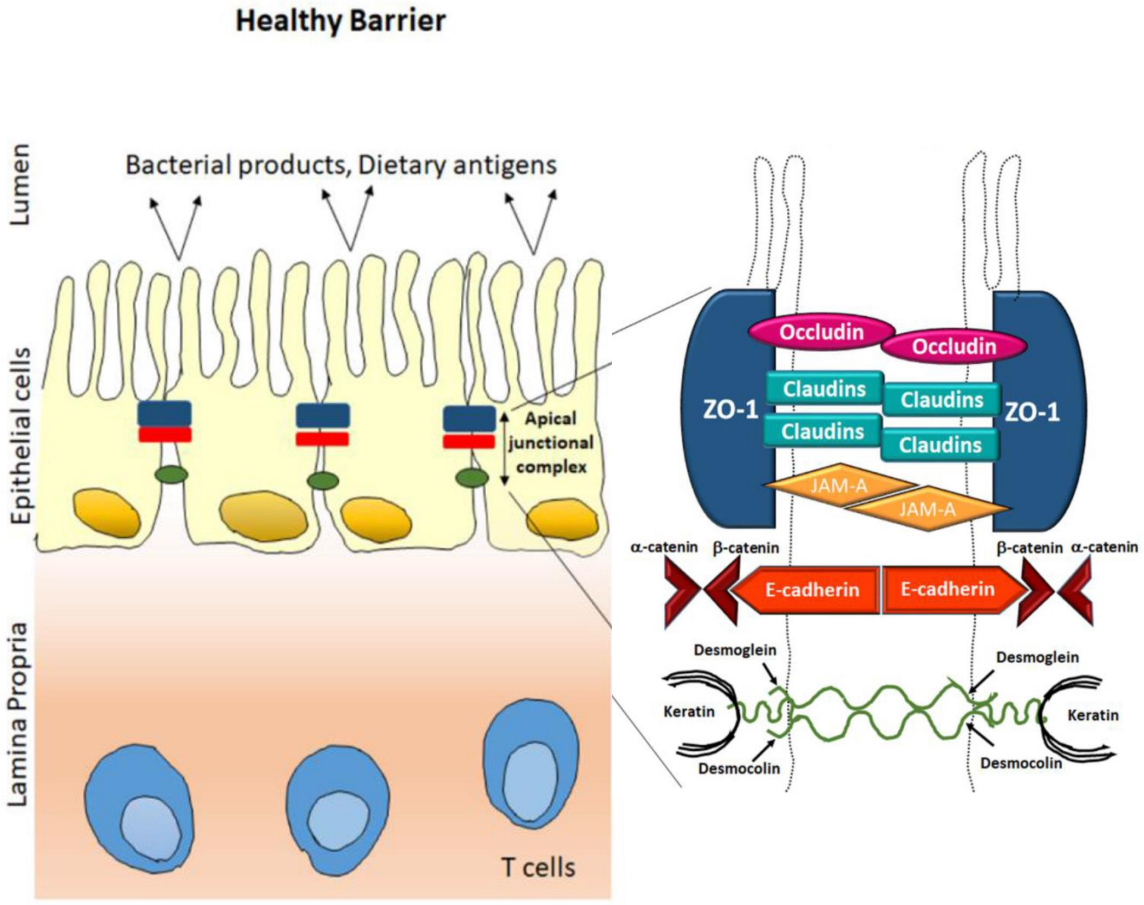
Microbiota over human life time

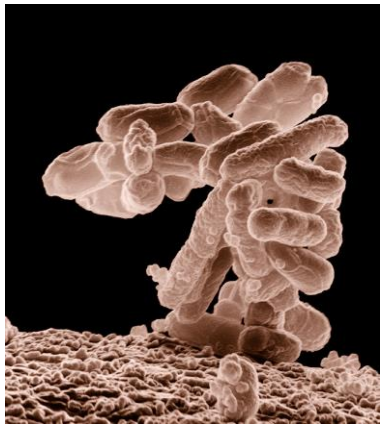


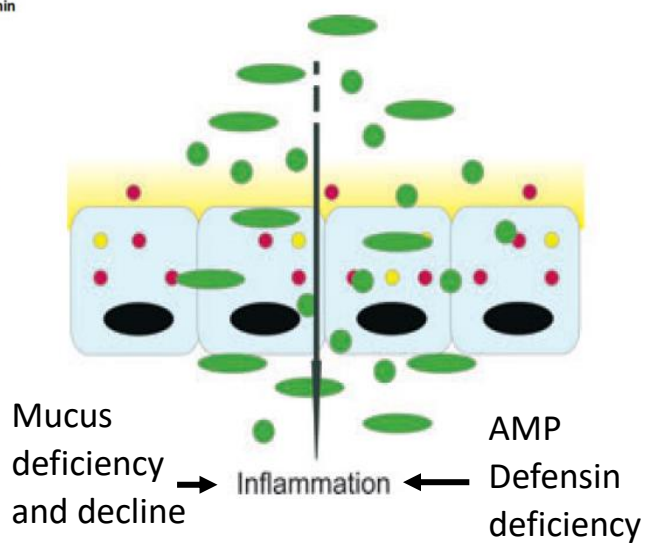
Colonization of neonatal calf rumen, post partum up to the first 12 weeks of life



Barrier integrity in maintaining a healthy gut







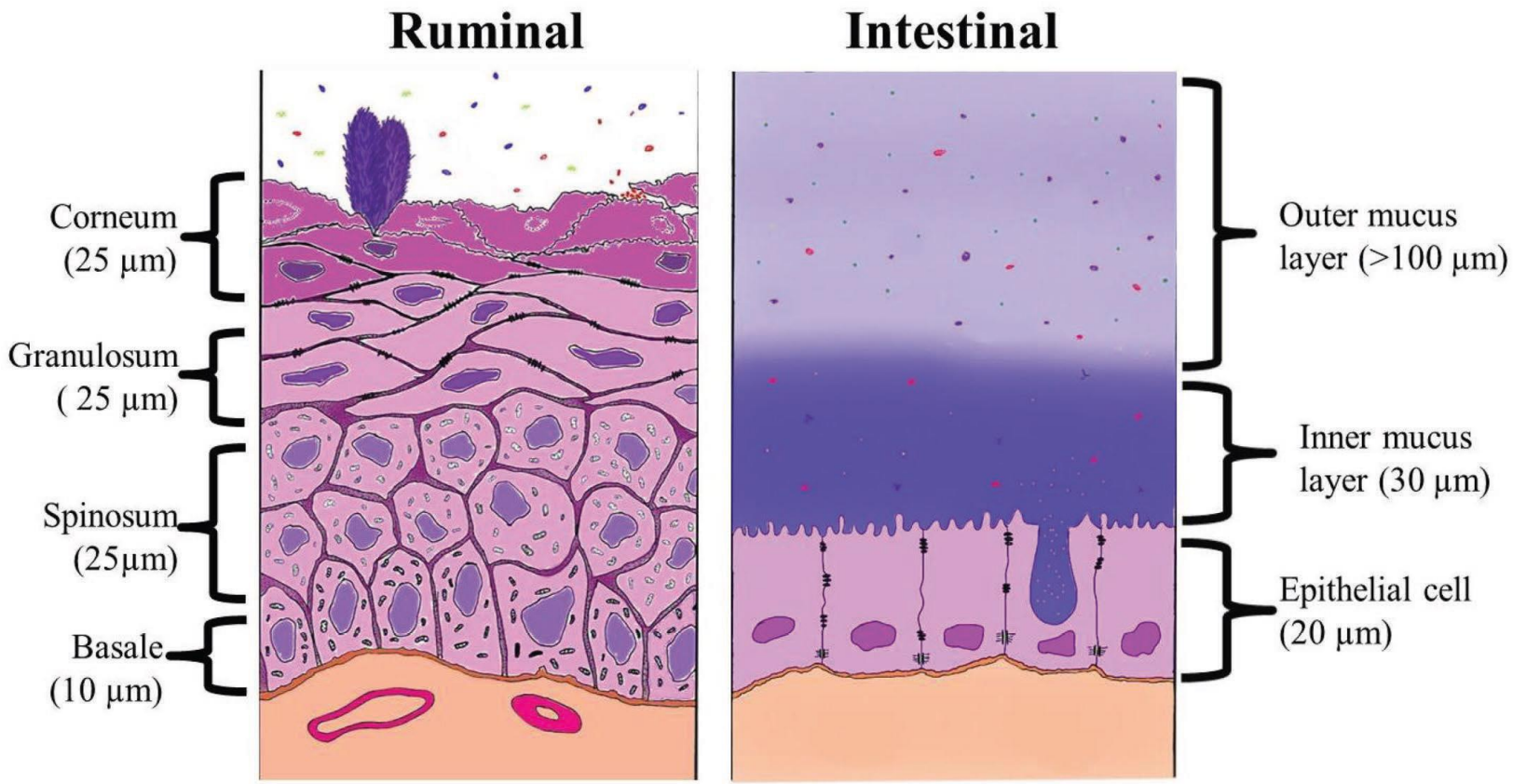
Panwar et al. Front. Physiol. 12:715611; 2021

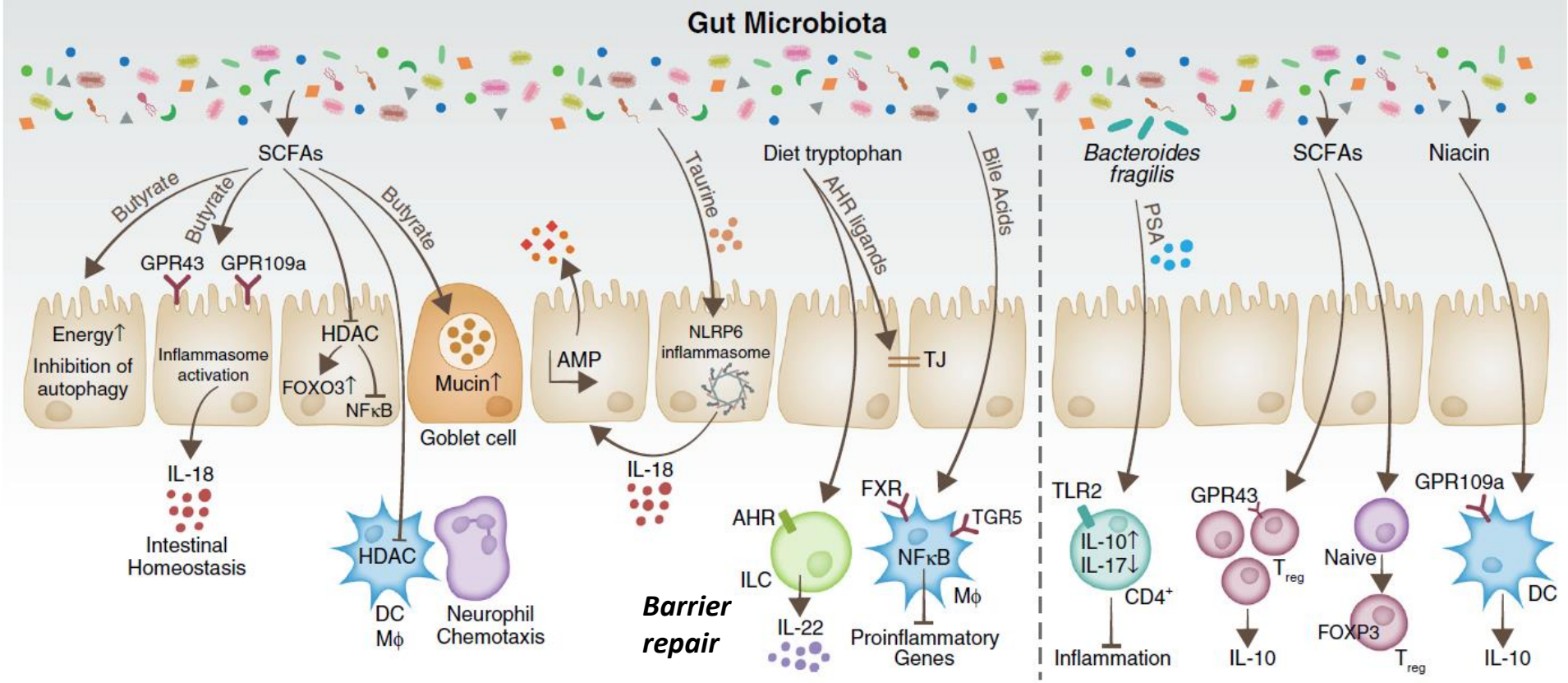
J. Dairy Sci. 99:4955–4966
http://dx.doi.org/10.3168/jds.2015-10351

Development and physiology of the rumen and the lower gut: Targets for improving gut health[1]

Michael A. Steele,*[2] Greg B. Penner,† Frédérique Chaucheyras-Durand,‡ and Le Luo Guan*

Stratified squamous epithelium ***Columnar epithelium***





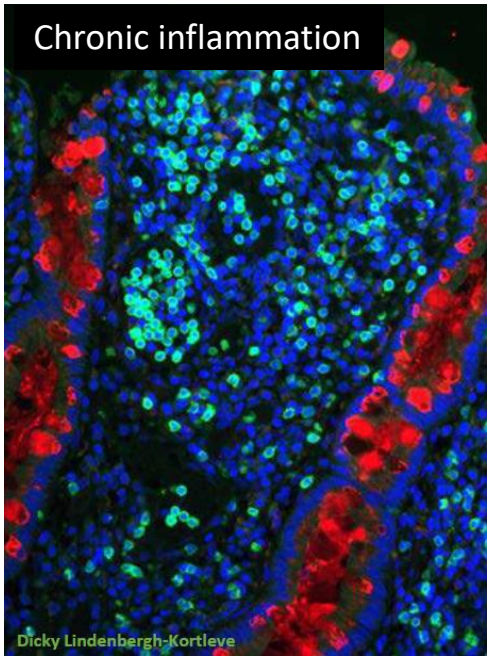
Unhealthy microbiota

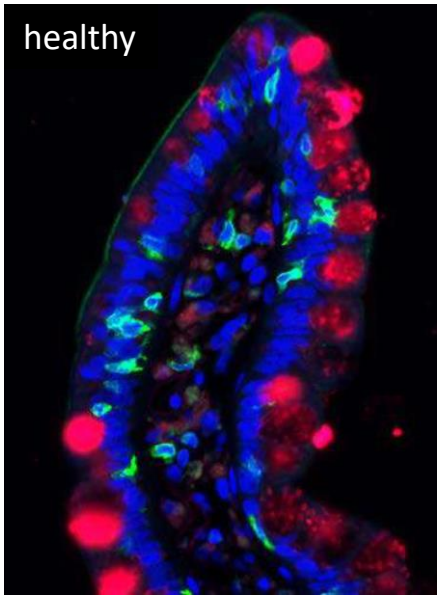
Modulation of immune signaling through microbial metabolites

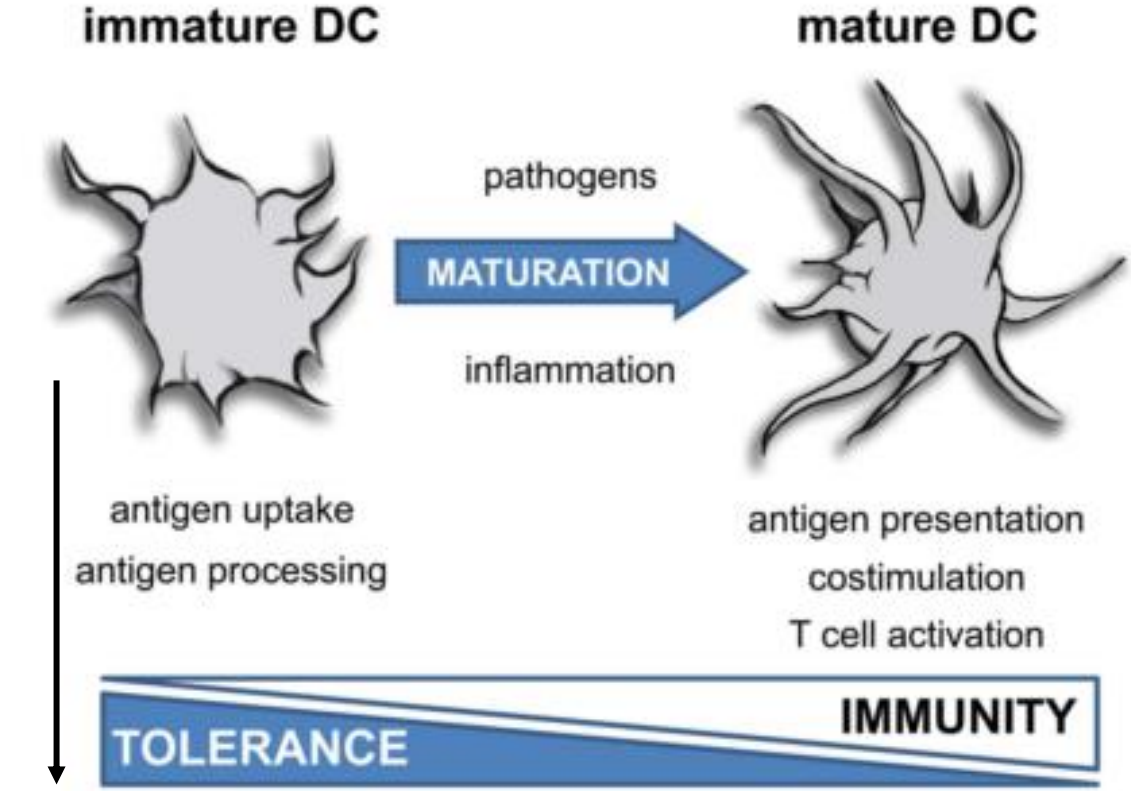
Levy et al. 2017

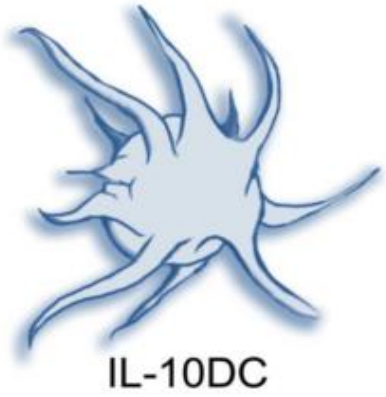
Healthy microbiota

DCs: gatekeepers of the mucosal immune system



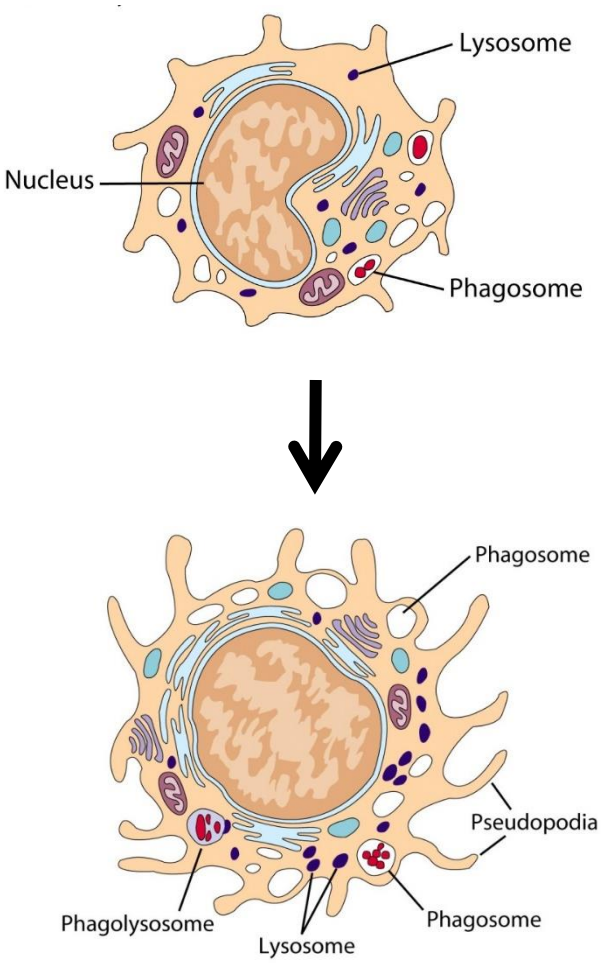


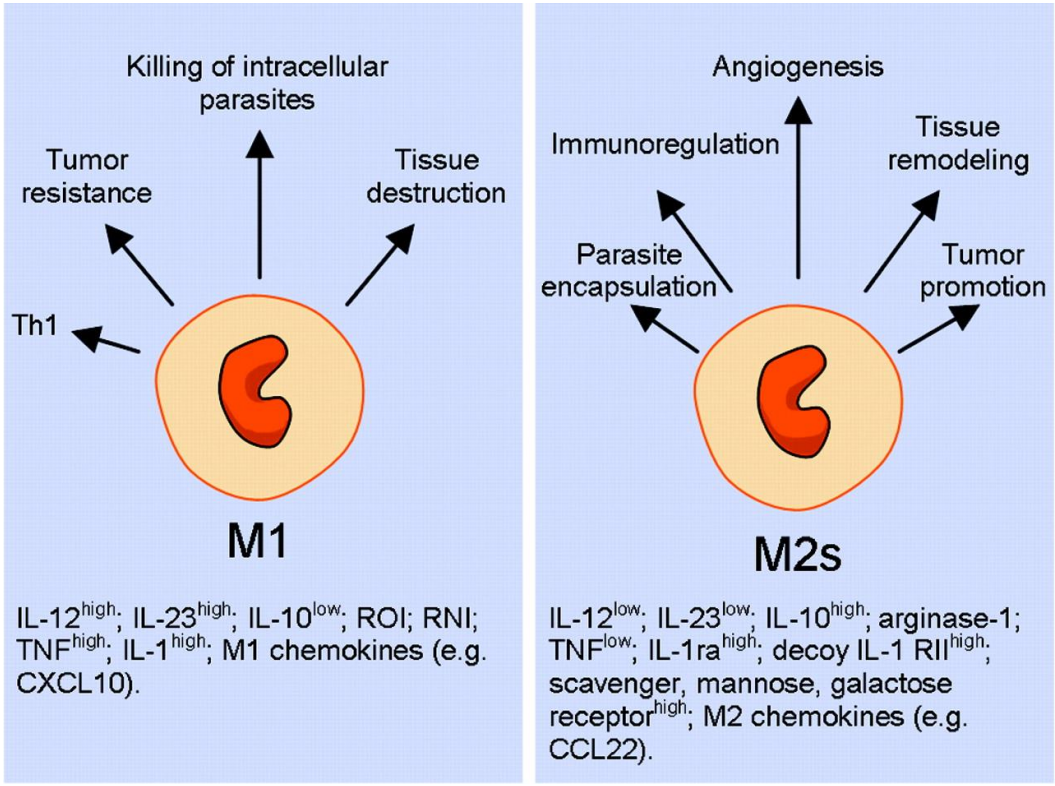




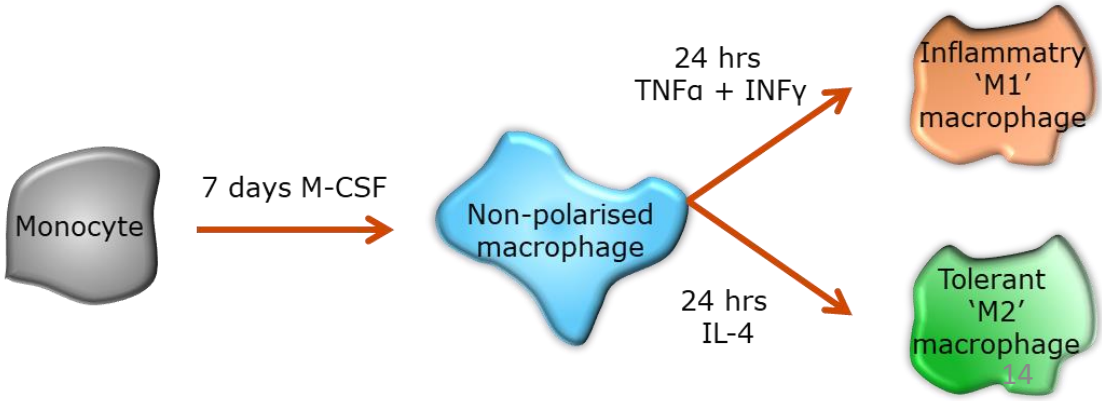
Key feature:
protects against infections, while avoiding destructive inflammatory response to commensal bacteria/dietary compounds.

Monocytes and macrophages for IM





Inflammation: injury **Fibrosis: repair**

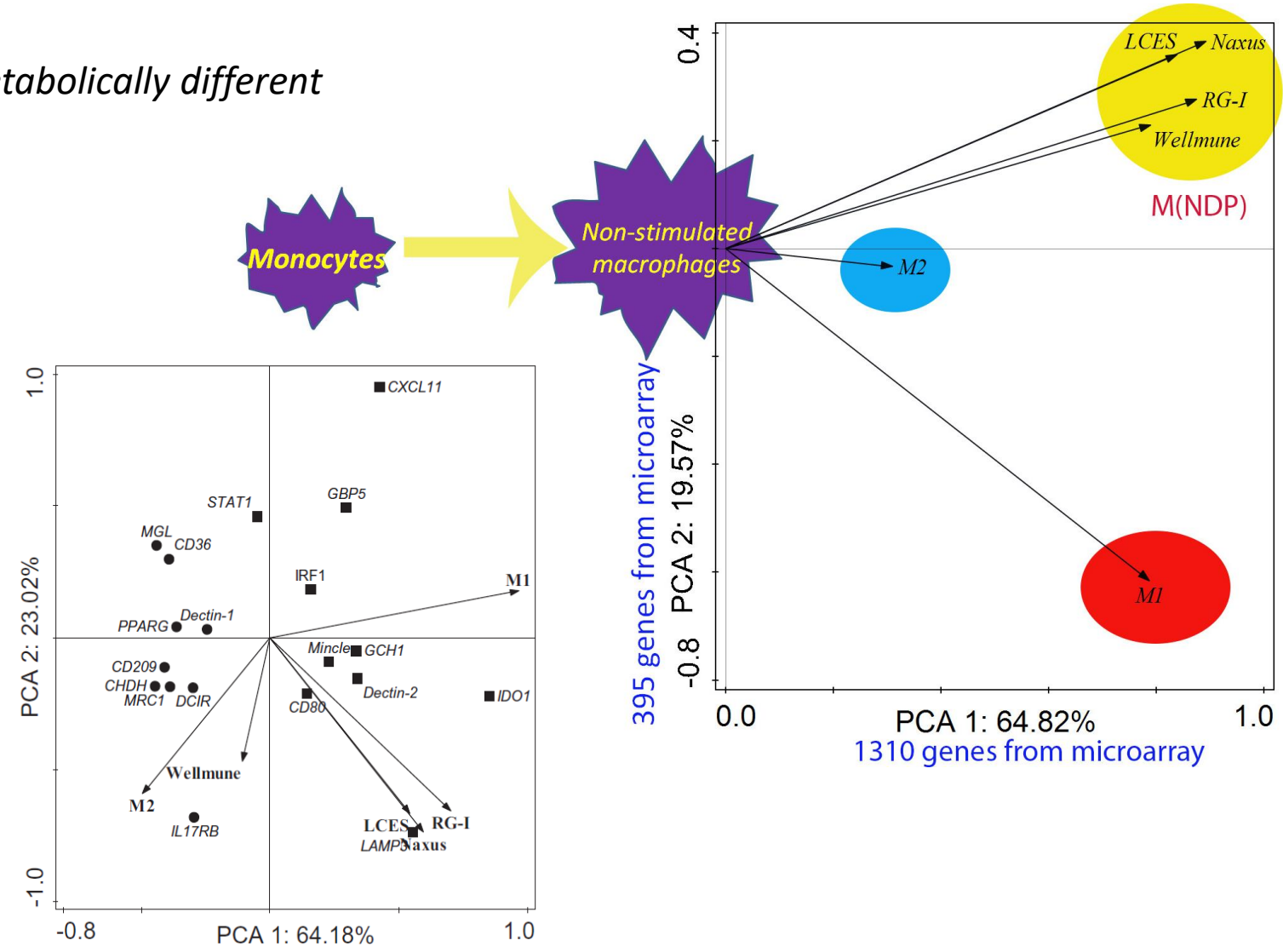


Macrophages treated with non-digestible polysaccharides reveal a transcriptionally unique phenotype

Journal of Functional Foods 36 (2017) 280–289

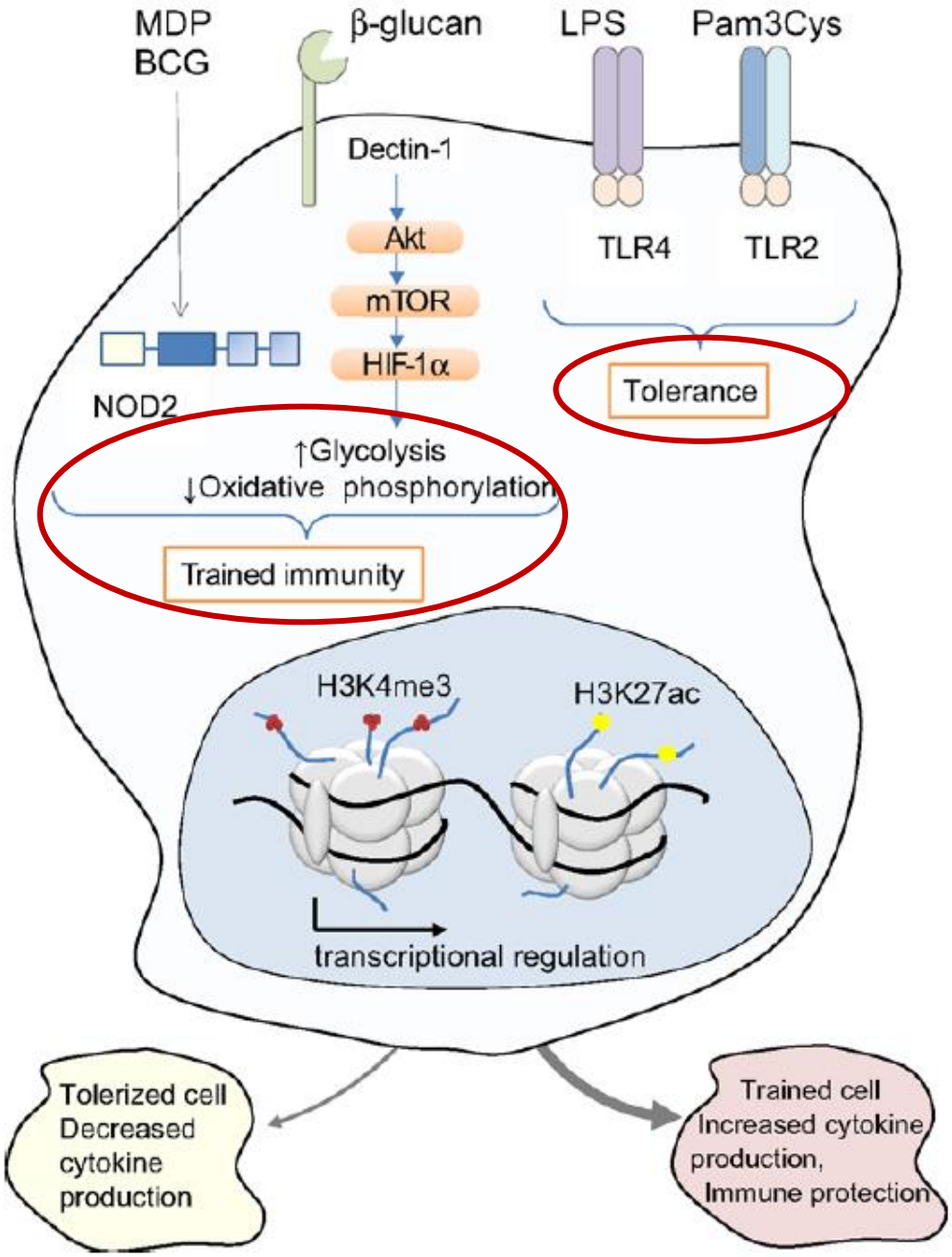
Yongfu Tang [a,b,c,1], Coen Govers [a,*,1], Harry J. Wichers [a,b], Jurriaan J. Mes [a]

Metabolically different



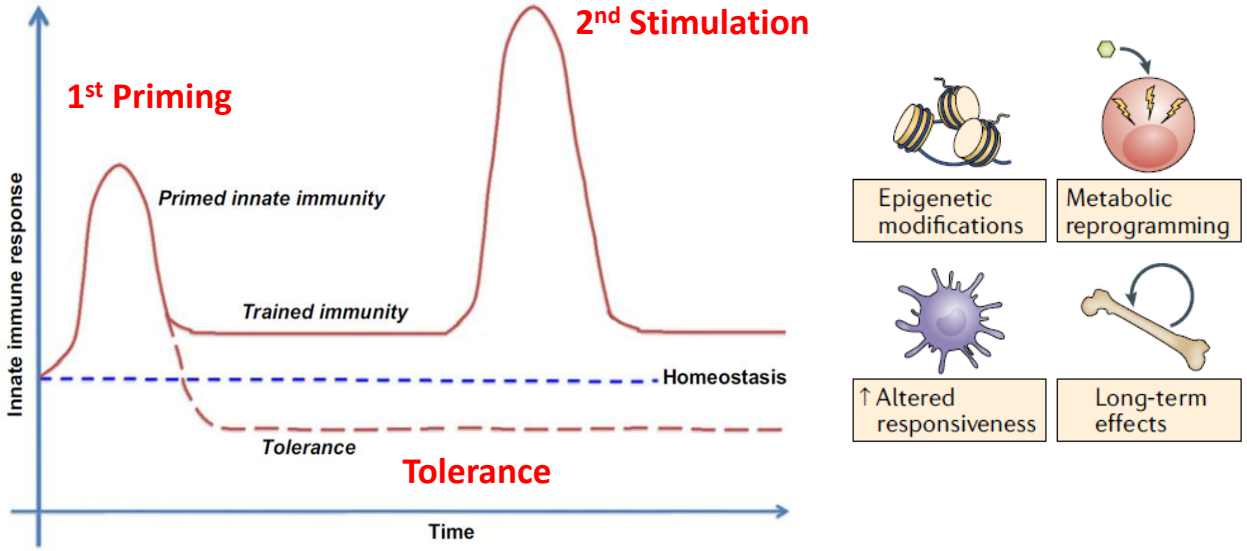
Microbial PAMP ligands are recognized by PRRs on innate immune cells and have long-term effects on the functional programme of the cell

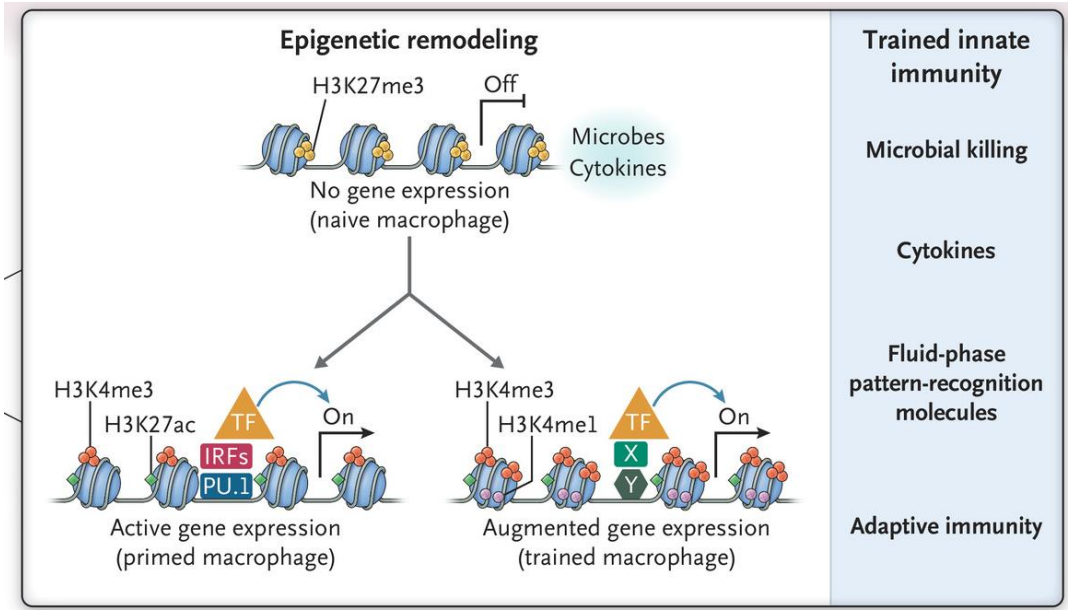
histone marks associated with open chromatin, such as trimethylation of lysine 4 residue of histone 3 (H3K4me3), H3K4me1, and H3K27Ac



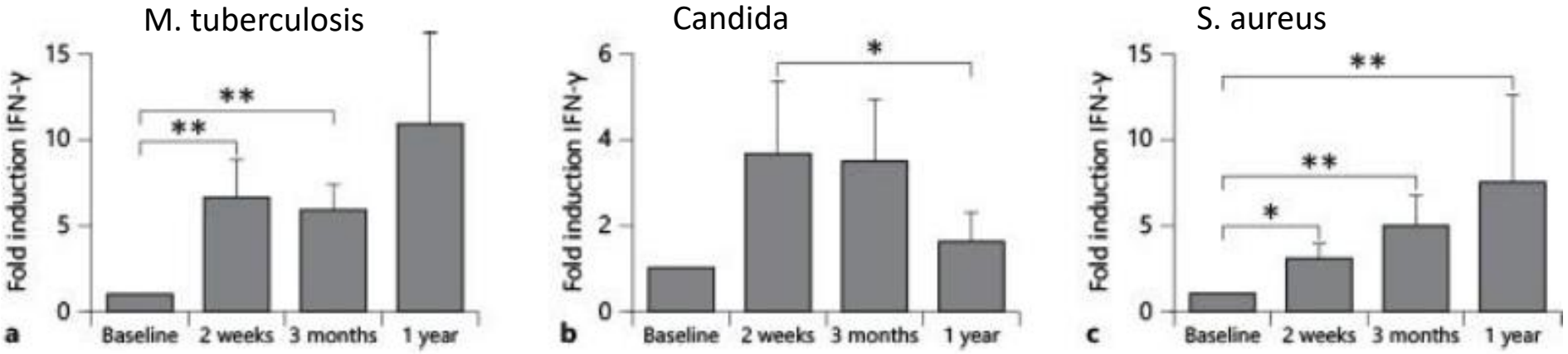
Defining trained immunity and its role in health and disease

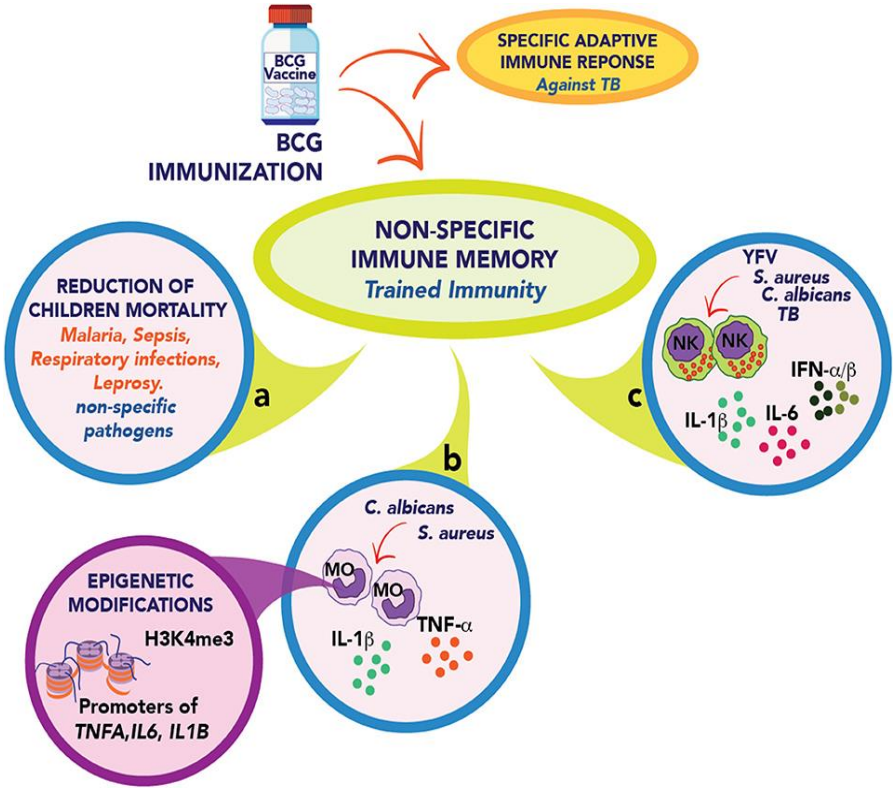
Mihai Netea et al. Nat Rev Immunol. 20: 375; 2020





BCG (innate) priming increases heterologous Th1 responses





Bacillus Calmette–Guérin (BCG). An attenuated form of the bacterium *Mycobacterium bovis*, which is the causative agent of bovine tuberculosis. This is now a vaccine to prevent tuberculosis (BCG vaccine), but it also induces protective heterologous effects against infections and malignancies.

Kleinnijenhuis et al, Clin Immunol 2014

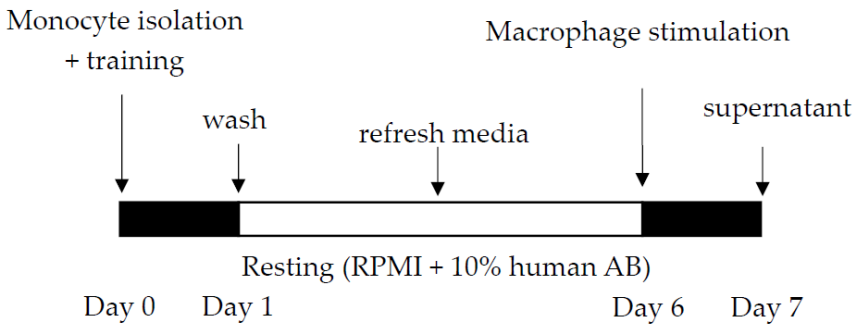
Induction of Trained Innate Immunity in Human Monocytes by Bovine Milk and Milk-Derived Immunoglobulin G

Nutrients 2018, *10*, 1378

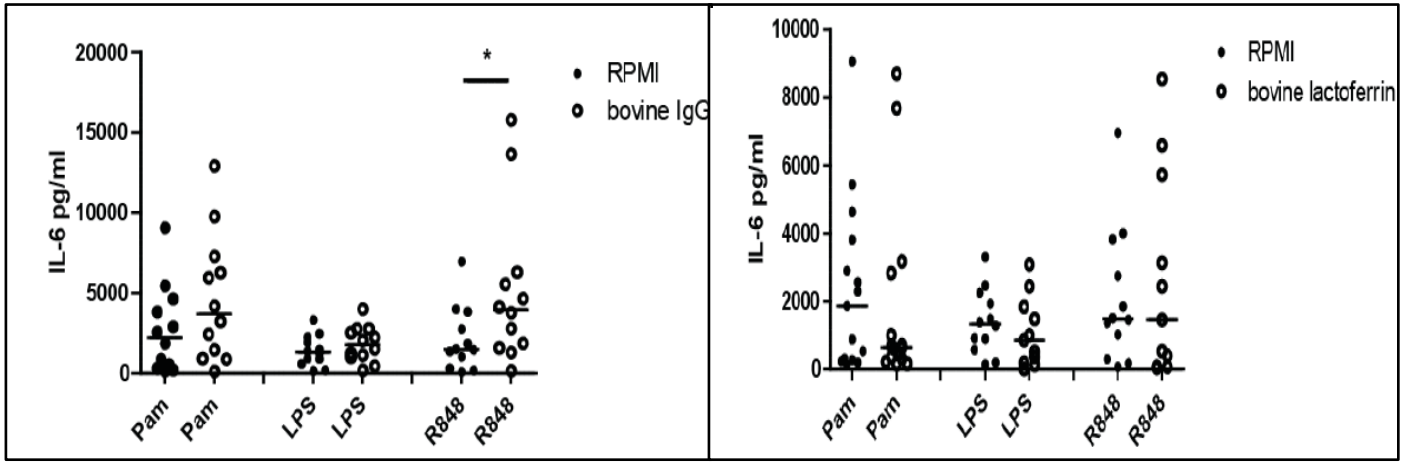
Marloes van Splunter [1], Thijs L. J. van Osch [1], Sylvia Brugman [1], Huub F. J. Savelkoul [1], Leo A. B. Joosten [2], Mihai G. Netea [2,3] and R. J. Joost van Neerven [1,4,*]

Component	Concentration	
Proteins	**Human milk (mg/mL)**	**Bovine milk (mg/mL)**
β-Casein	4.7	9.6
κ-Casein	1.4	3.4
α_{S1}-Casein	0.9	10.6
α_{S2}-Casein	Absent	2.8
β-Lactoglobulin	Absent	3.1
α-Lactalbumin	3.0	0.9
Serum albumin	0.5	0.3
Lactoferrin	2.0	0.1
Lysozyme	0.5	0.0004
Osteopontin	0.14	0.02
IgG	0.04	0.63

TLR	TLR-ligand
TLR1/2	Pam3Cysk4
TLR4	LPS
TLR7/8	R848



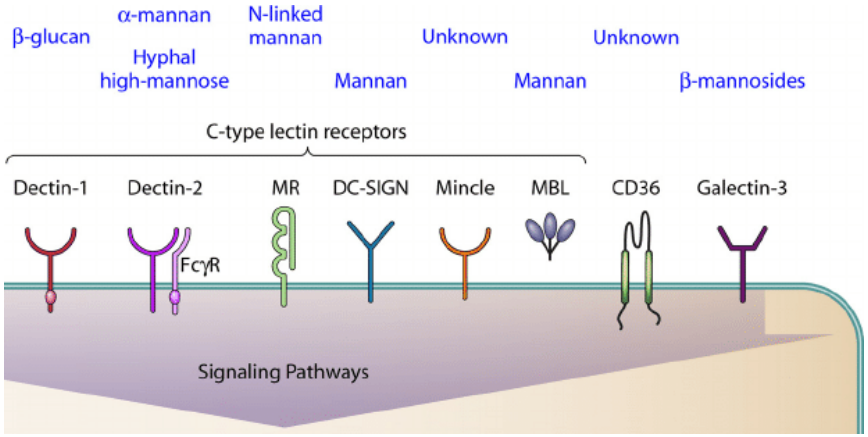
van Neerven, Knol, Heck, Savelkoul, JACI, 2012



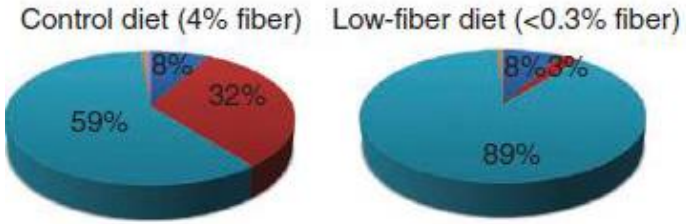
Continuous Exposure to Non-Soluble β-Glucans Induces Trained Immunity in M-CSF-Differentiated Macrophages

Front. Immunol. 12:672796; 2021.

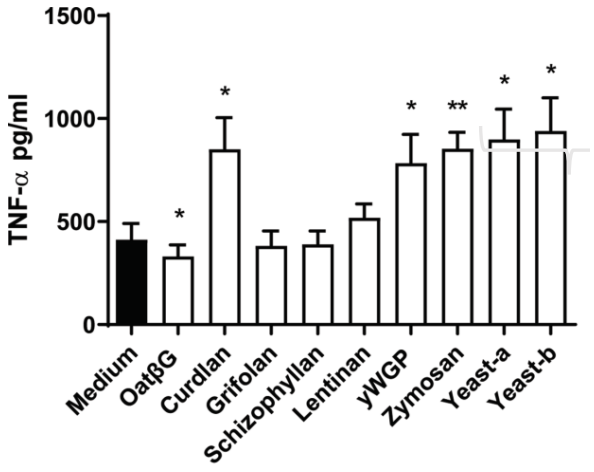
Bart G. J. Moerings, [1,2,†] Priscilla de Graaff, [1,3,†] Matthew Furber, [4] Renger F. Witkamp, [2] Reno Debets, [3] Jurriaan J. Mes, [1] Jeroen van Bergenhenegouwen, [4,‡] and Coen Govers [1,5,*,‡]

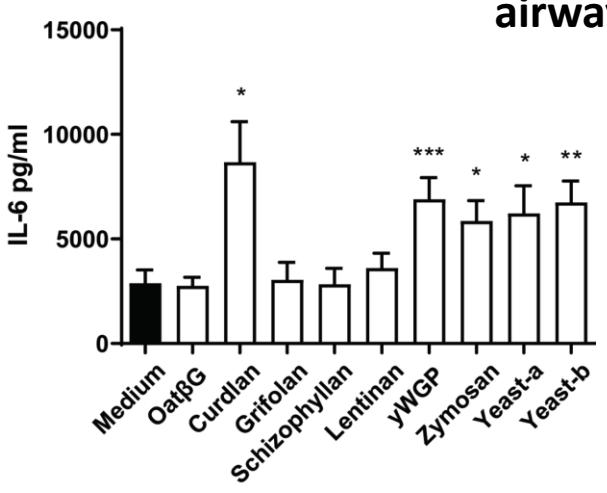


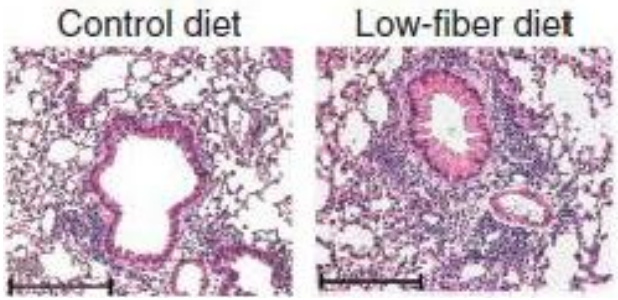
Mice on low fiber have less complex microbiota



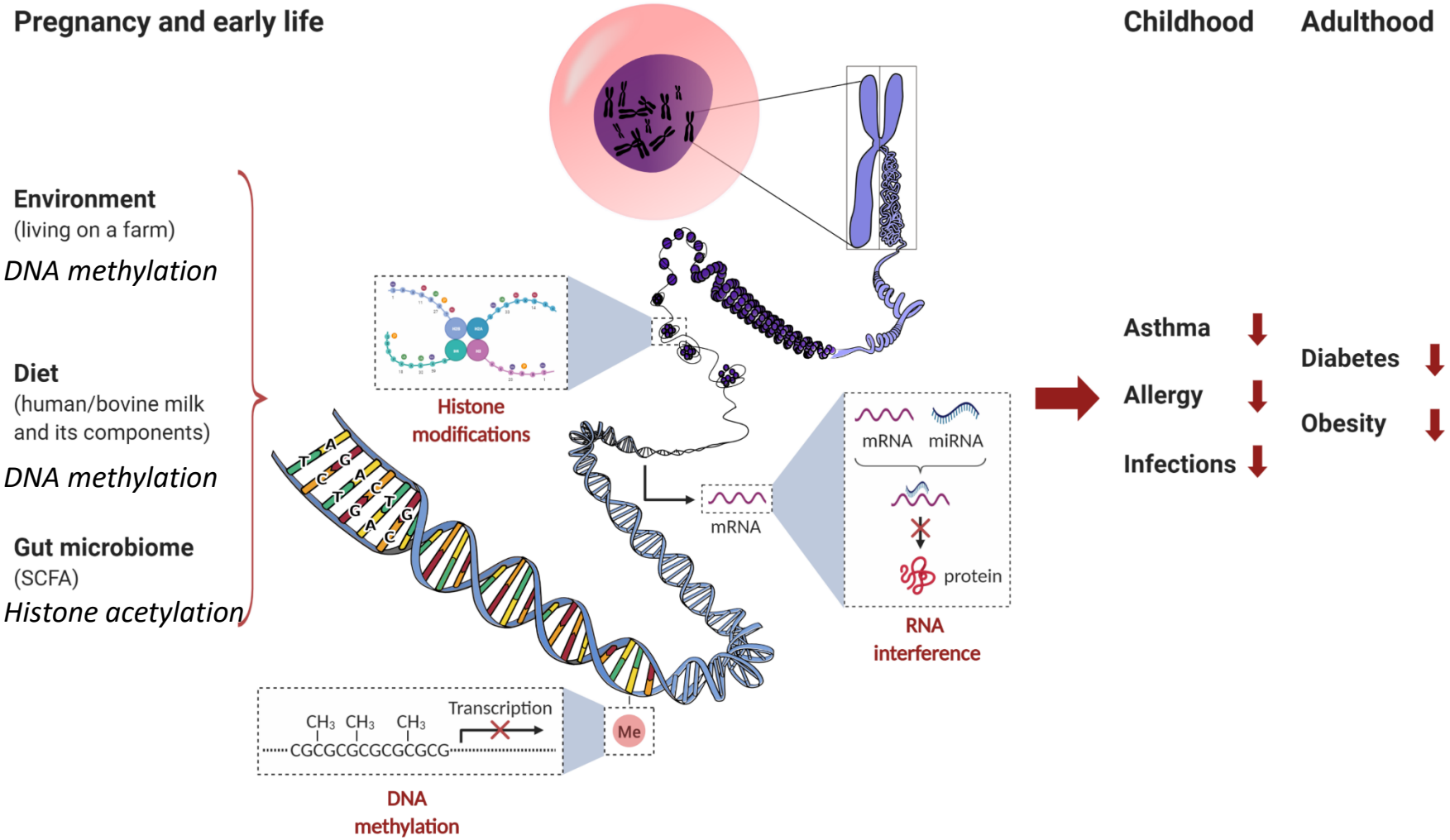
Mice on low fiber diet have increased airway inflammation

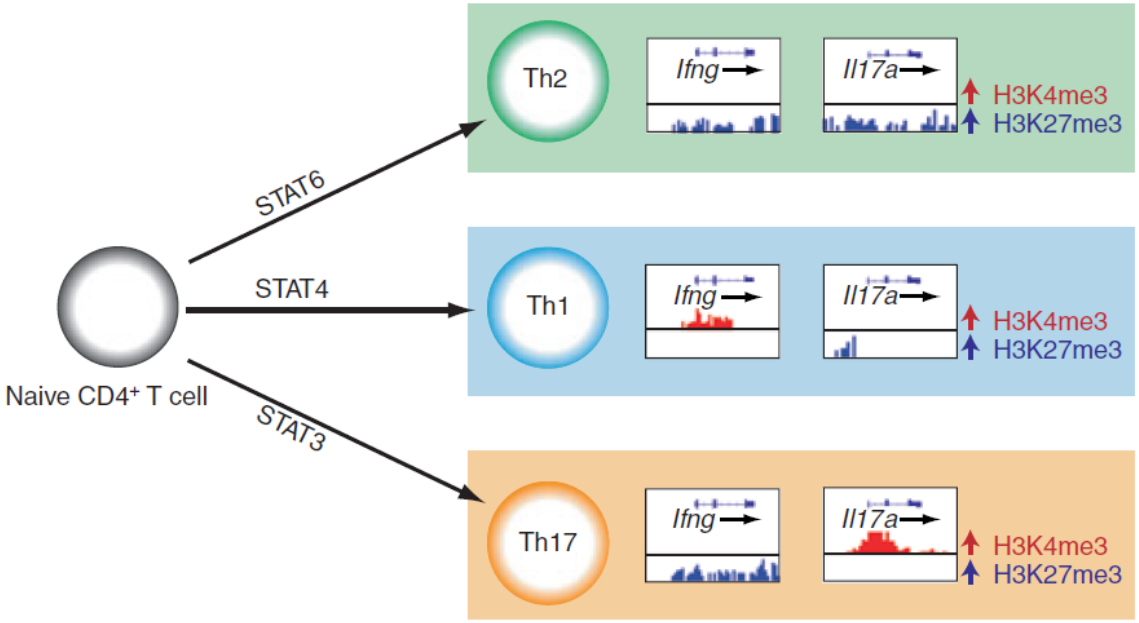






Diet-induced epigenetics modulate transcriptional activity with long-lasting consequences in the newborn



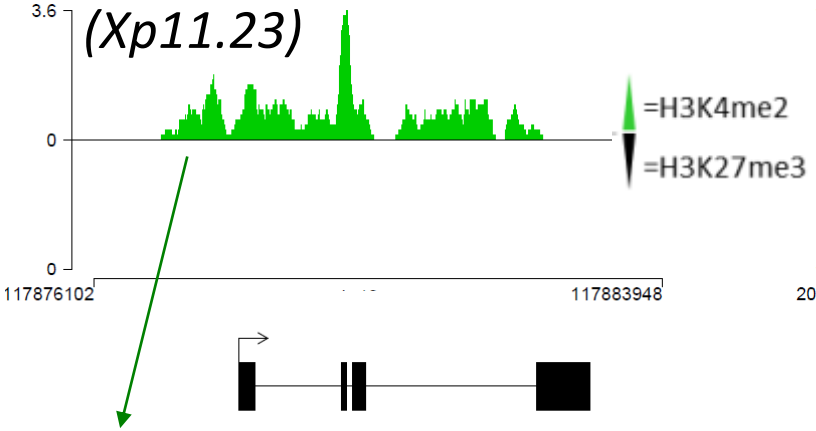


Histone modification	Histone mark(s)
Acetylation (H3, H4)	H3K9ac, H3K14ac, H3K18ac
	H3K14ac, H3K18ac, H4K5ac, H4K8ac
Methylation	H3K4me1
	H3K4me2, H3K4me3
	H3K27me2, H3K27me3
	H3K9me2, H3K9me3
Phosphorylation	H3S10p

Chromatin immunoprecipitation-sequencing (ChIP-seq)

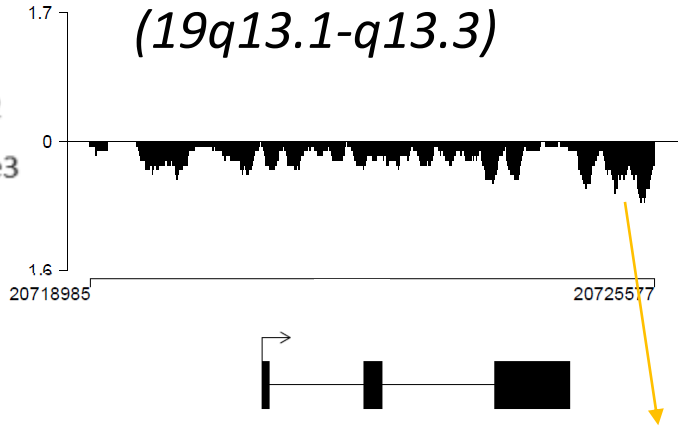
CD4+ T-cells

Gene Foxp3 induced (Xp11.23)



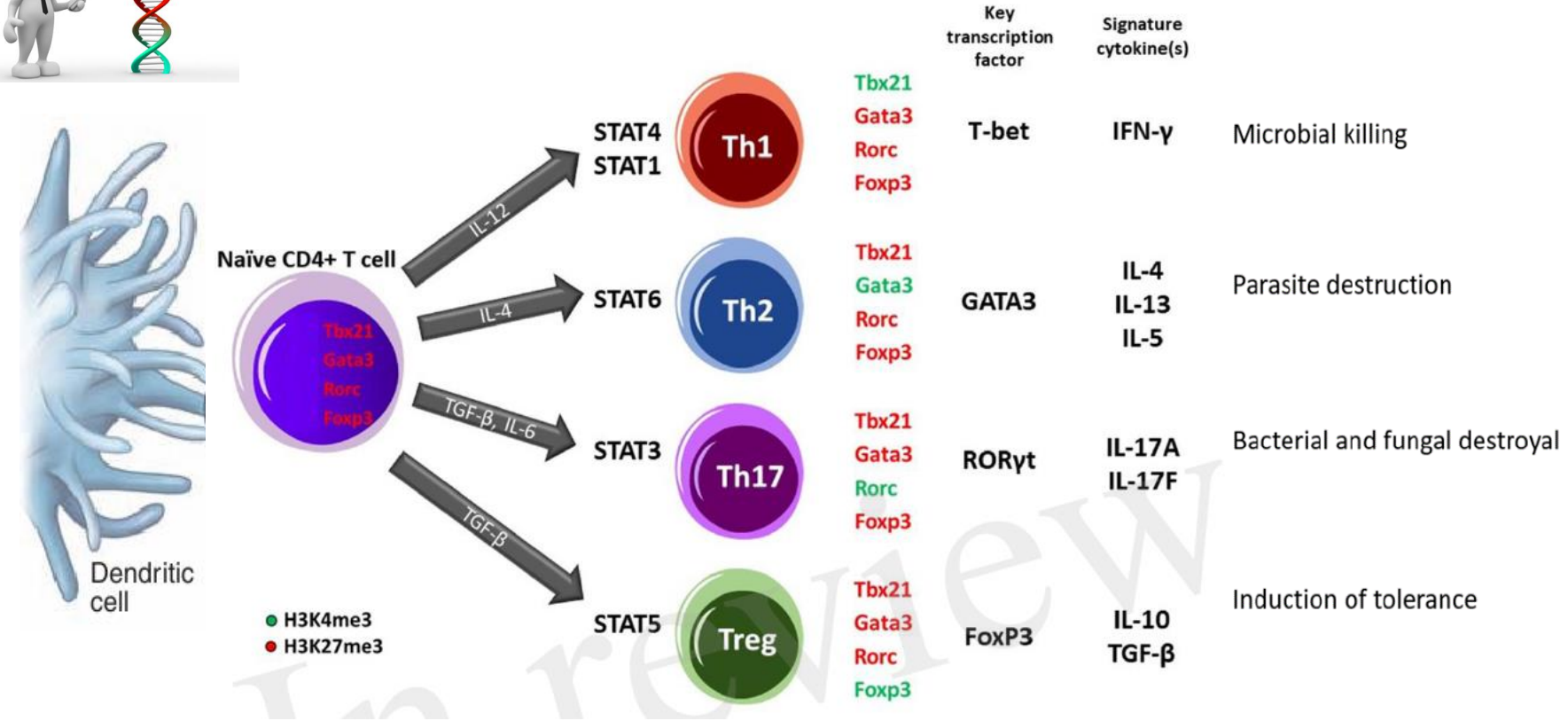
H3K4me2 → *active*

Gene TGF-β removed (19q13.1-q13.3)



H3K27me3 → *repressive*

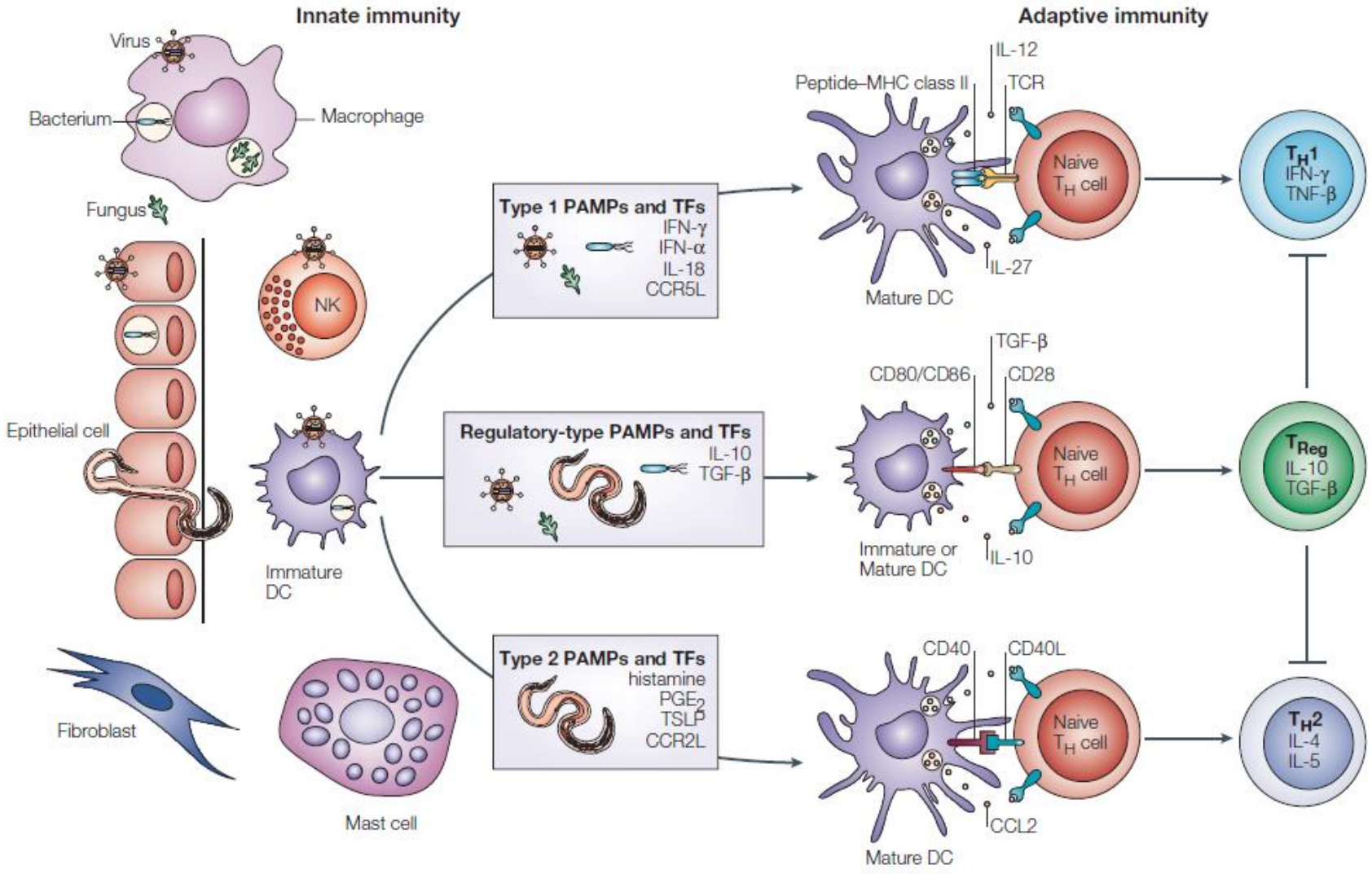
T-cell subsets



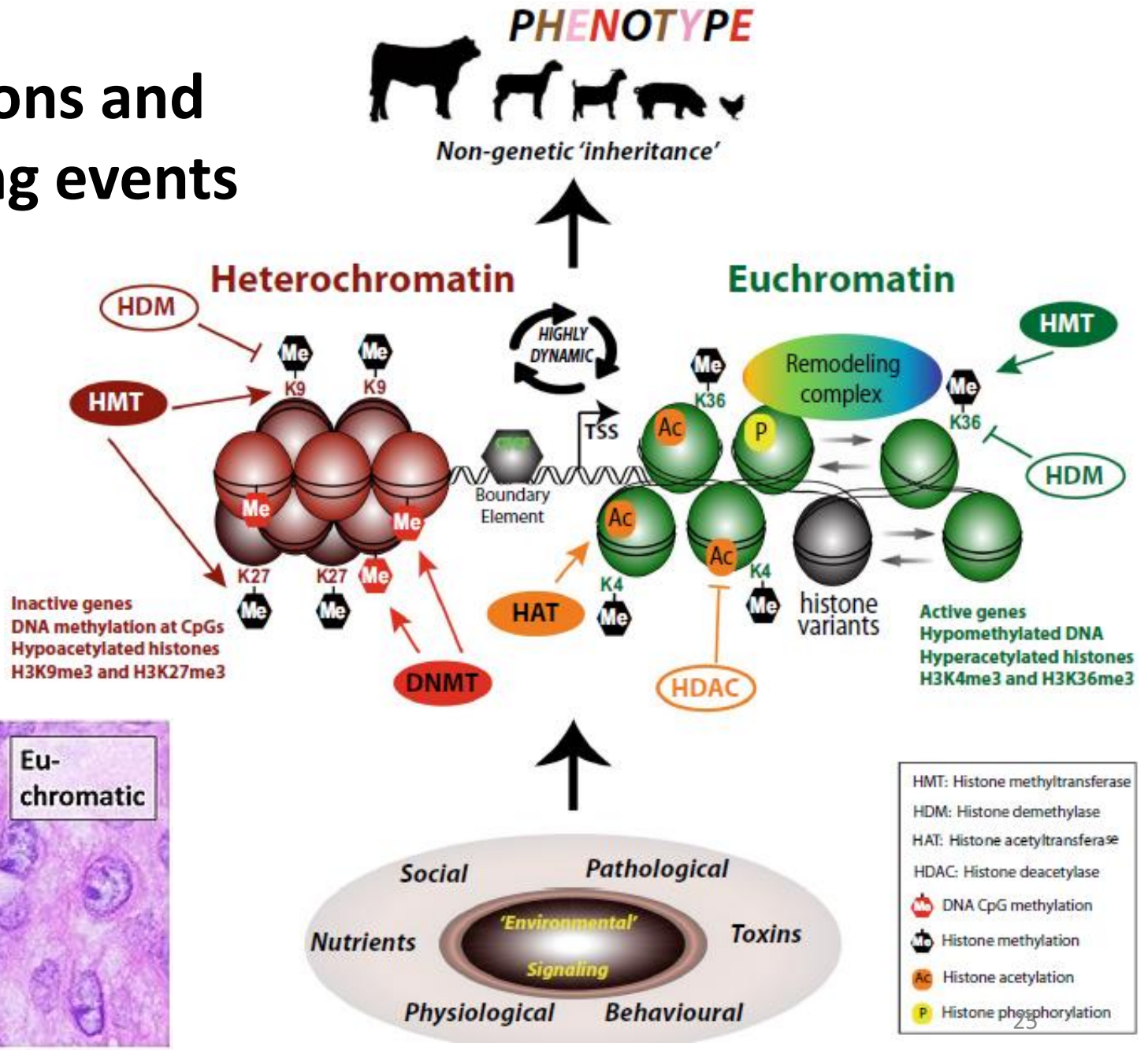
Unlike genetic factors, epigenetic marks are reversible, allowing reprogramming after birth by several factors, including nutrition, infection, stress, housing conditions,...
Nutrition-immunity cross talk through epigenetic regulation is now offering opportunities for prevention and treatment of human diseases from chronic noncommunicable diseases to brain and behavioural disorders. This now awaits application in the bovine system!

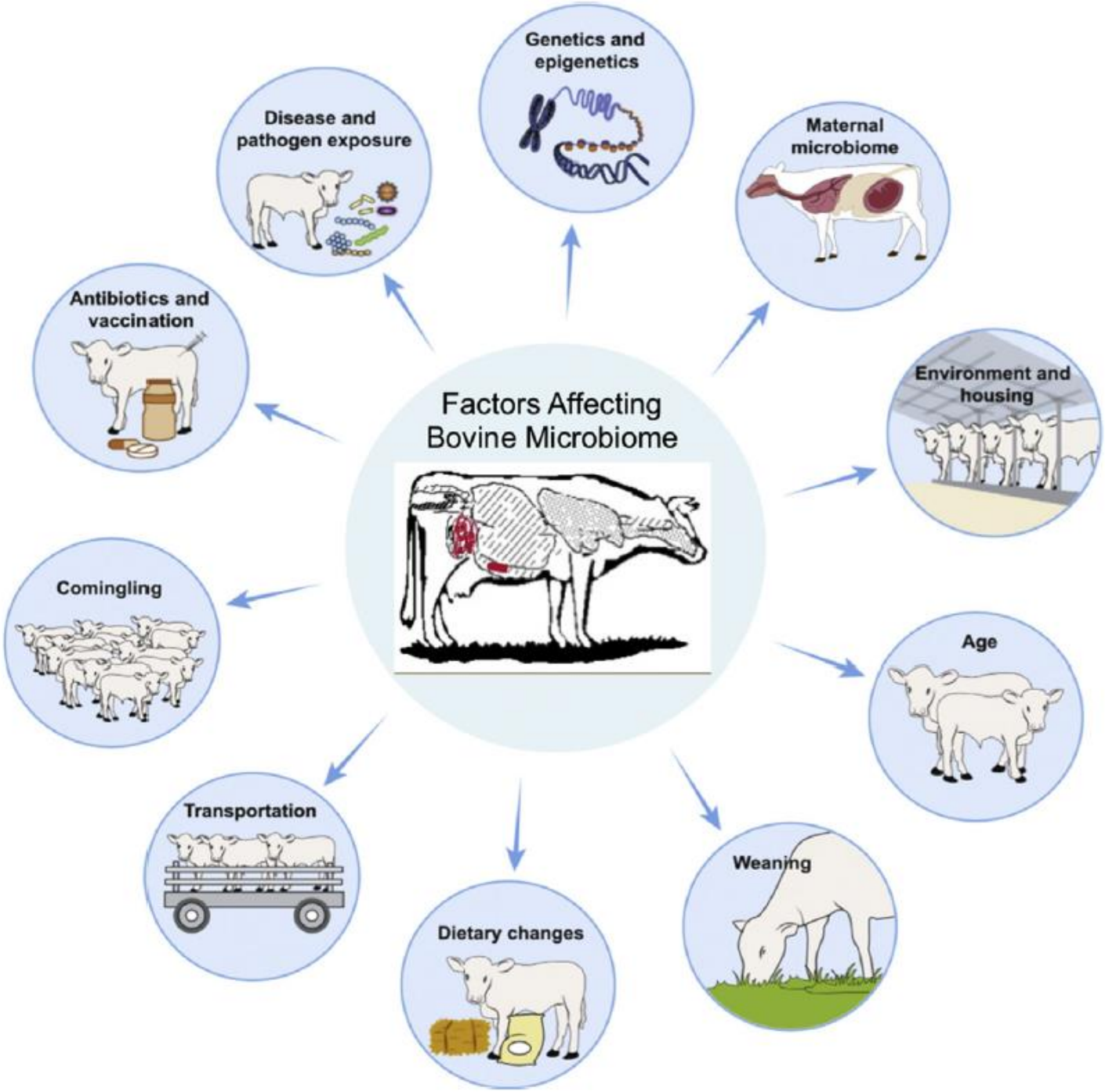
van Esch et al, Front. Immunol. 11:2141; 2020

DC polarization directed by type of micro-organism that is recognised and the site of activation

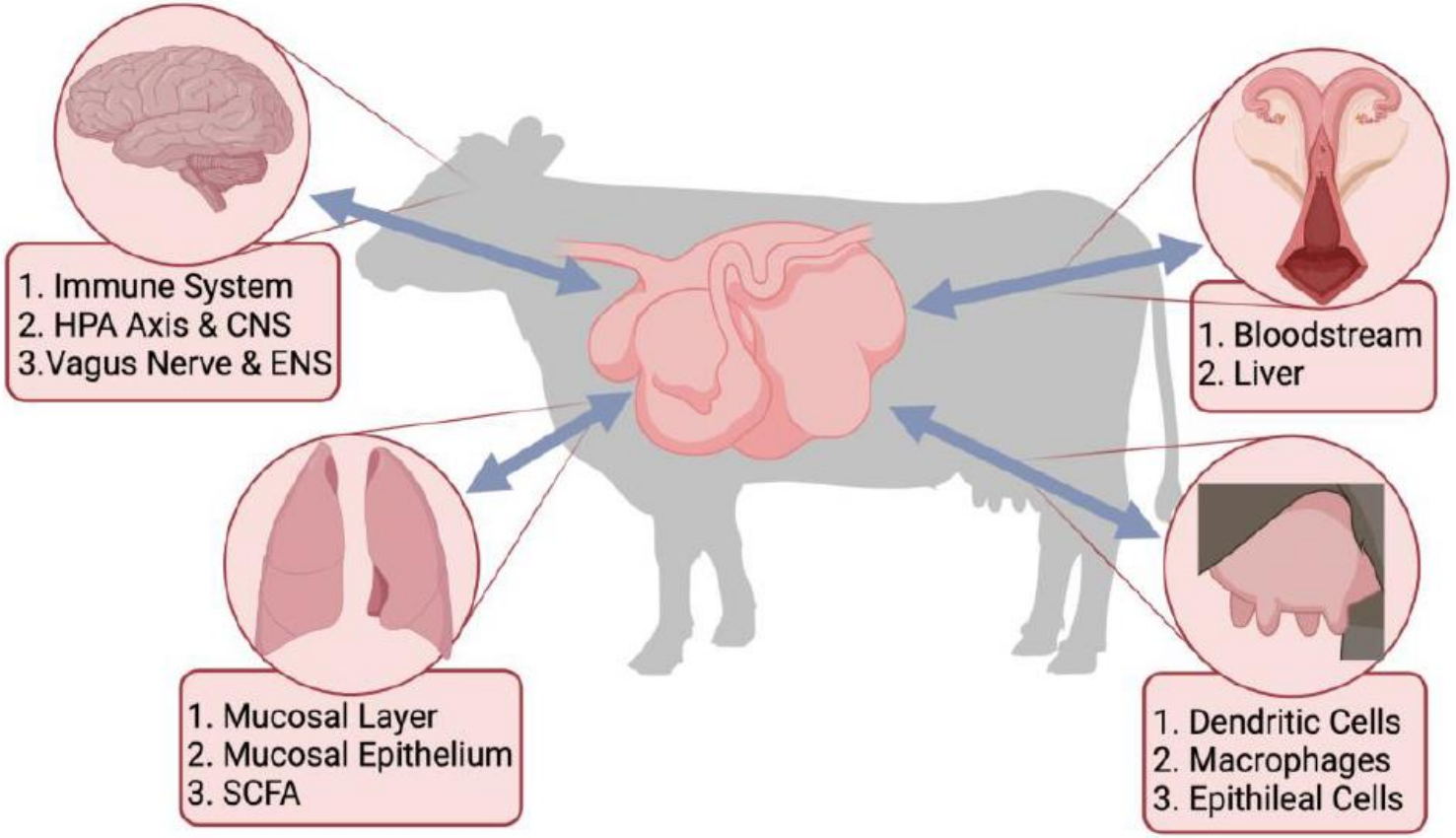


Chromatin modifications and remodelling events




Genetics and epigenetics
Disease and pathogen exposure
Maternal microbiome
Antibiotics and vaccination
Factors Affecting Bovine Microbiome
Environment and housing
Comingling
Age
Transportation
Weaning
Dietary changes

Proposed links between the gastrointestinal tract microbiota and different organ systems through the microbiome-gut-organ axes



microorganisms

MDPI

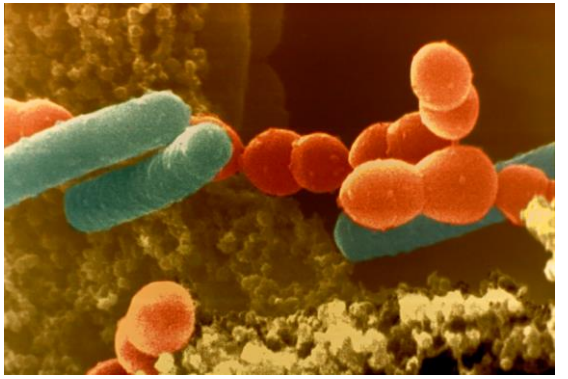
Review

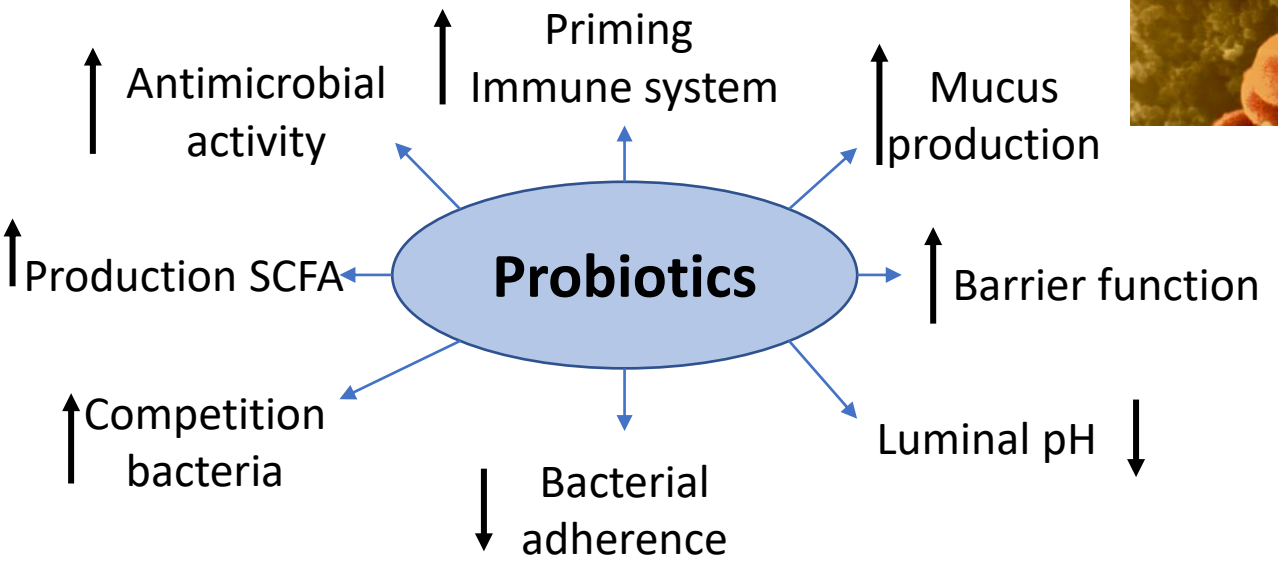
Utilizing the Gastrointestinal Microbiota to Modulate Cattle Health through the Microbiome-Gut-Organ Axes

Christina B. Welch *, Valerie E. Ryman, T. Dean Pringle and Jeferson M. Lourenco

2022, 10, 1391.
https://doi.org/10.3390/microorganisms10071391

Probiotics modulate microbiota composition and host functions





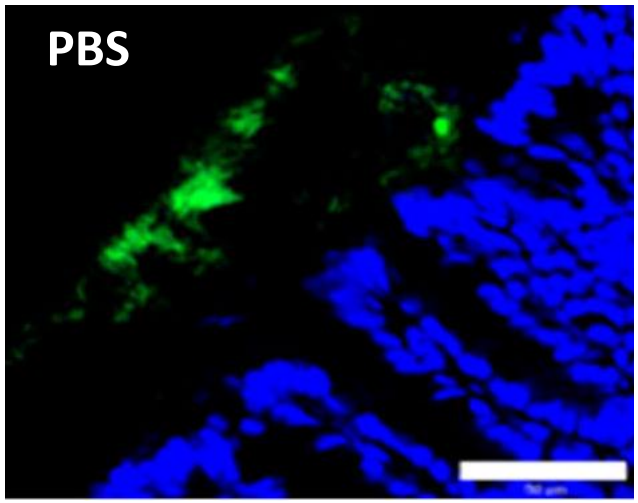
Bulgarian yoghurt contains *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and regular consumption prevents “pollution” of the gut and provides longer and healthy life (Eli Metchnikoff, Nobel prize 1908)

Probiotics: living micro-organisms that when administered in adequate amounts and in active (life) form reach the gut, will provide a health-promoting efect. (EFSA)

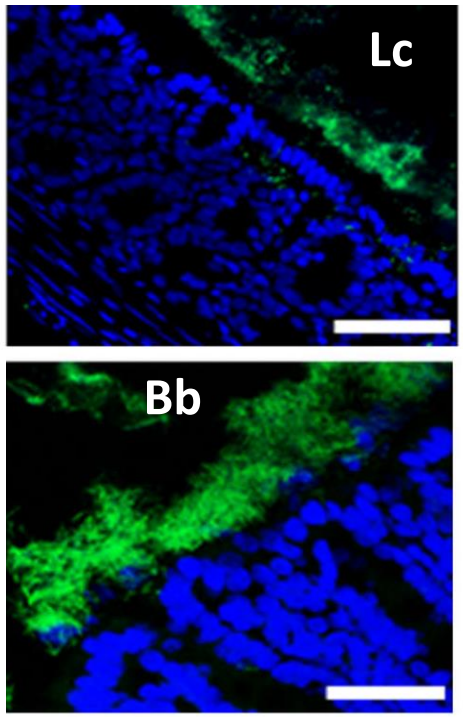
Supplementation with *Lactobacillus plantarum* WCFS1 Prevents Decline of Mucus Barrier in Colon of Accelerated Aging *Ercc1*$^{-/\Delta 7}$ Mice

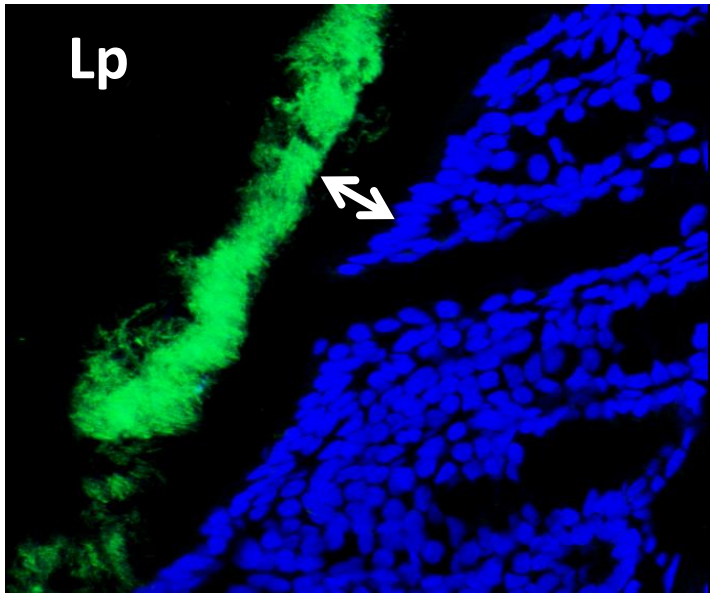
Front. Immunol. 7:408; 2016.

Adriaan A. van Beek[1,2,3†], Bruno Sovran[2,4], Floor Hugenholtz[2,5], Ben Meijer[1], Joanne A. Hoogerland[1], Violeta Mihailova[1], Corine van der Ploeg[1], Clara Belzer[2,5], Mark V. Boekschoten[2,6], Jan H. J. Hoeijmakers[7,8], Wilbert P. Vermeij[7], Paul de Vos[2,9], Jerry M. Wells[2,4], Pieter J. M. Leenen[10], Claudio Nicoletti[3,11], Rudi W. Hendriks[12] and Huub F. J. Savelkoul[1,2]*



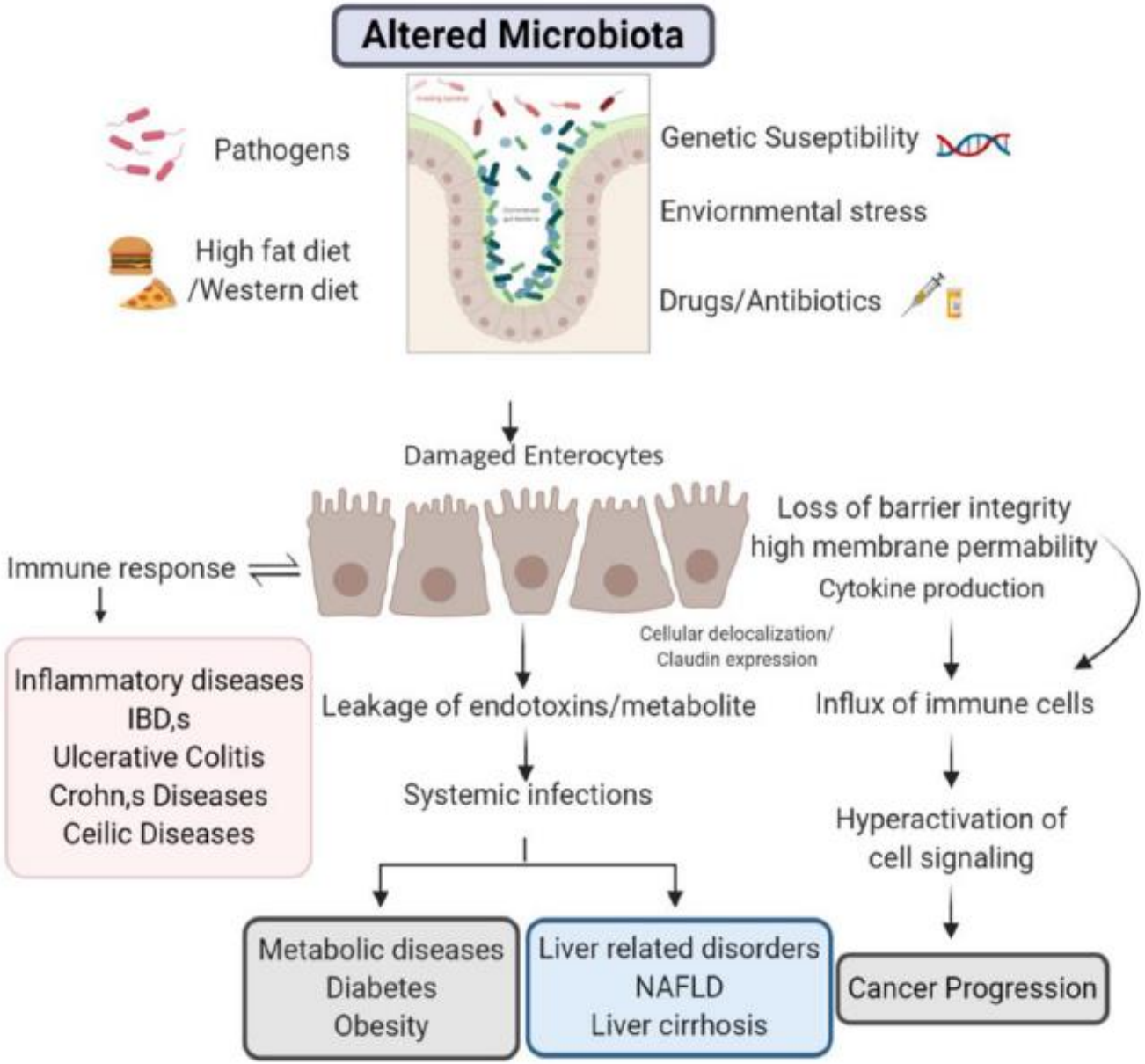
Old mouse



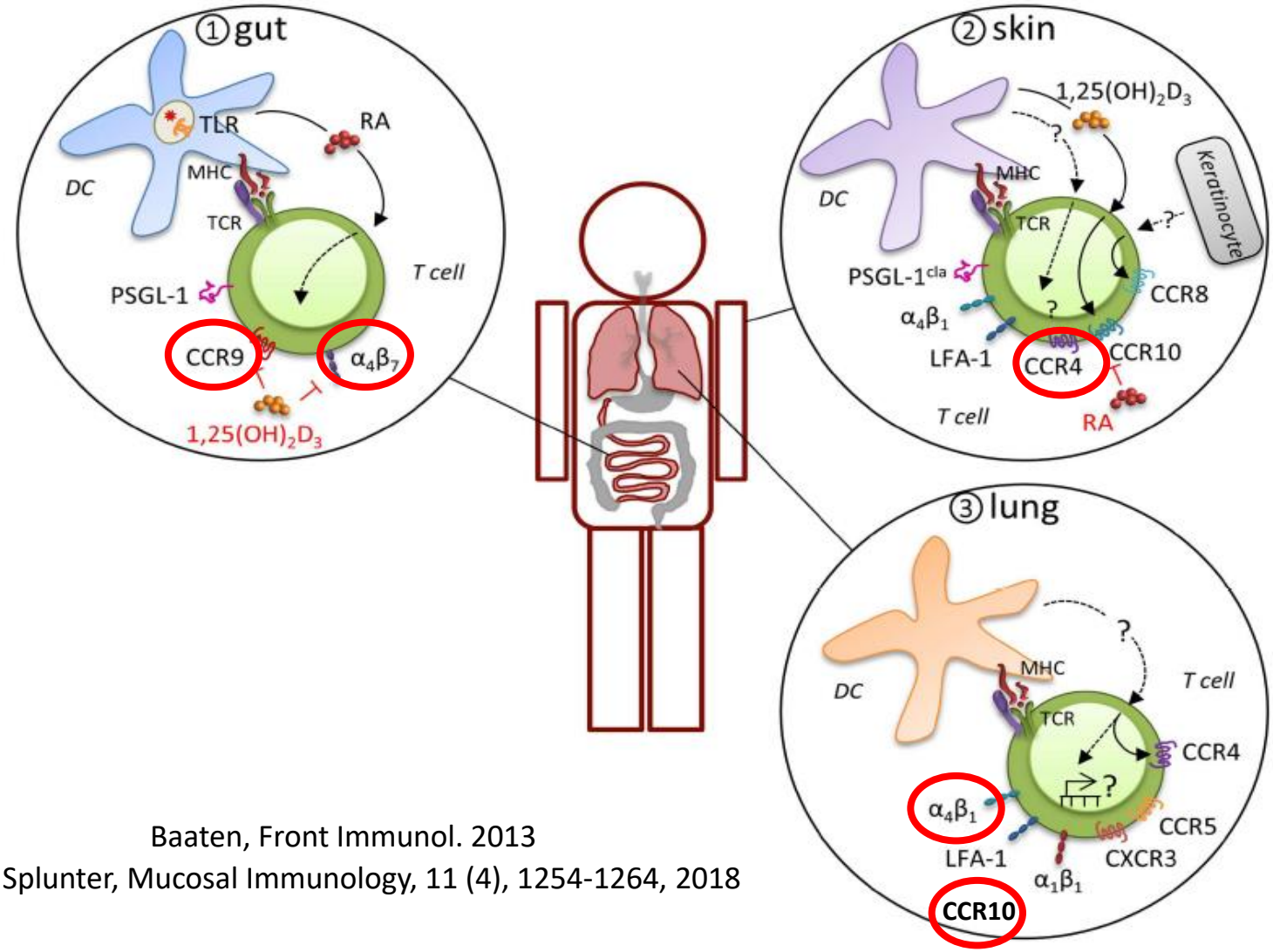


Old mouse plus probiotics
10 weeks, 3x per week

Conceptual relevance of gut-organ axis for health



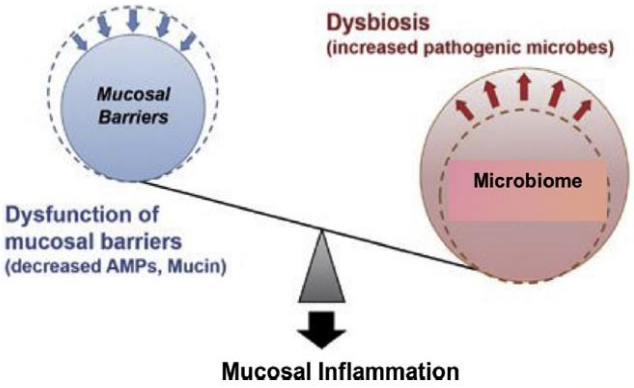
Tissue specific homing of immune cells by chemokines and their receptors

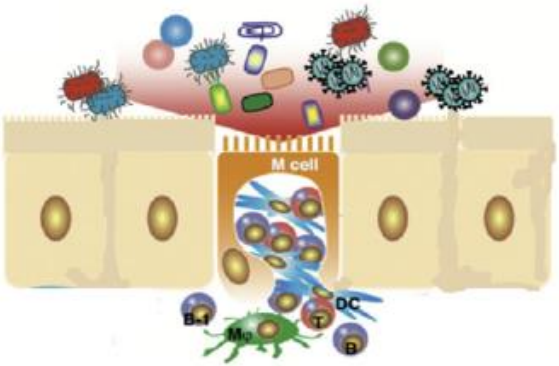


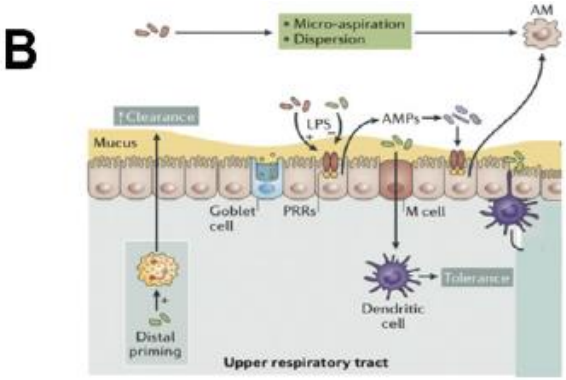
Baaten, Front Immunol. 2013

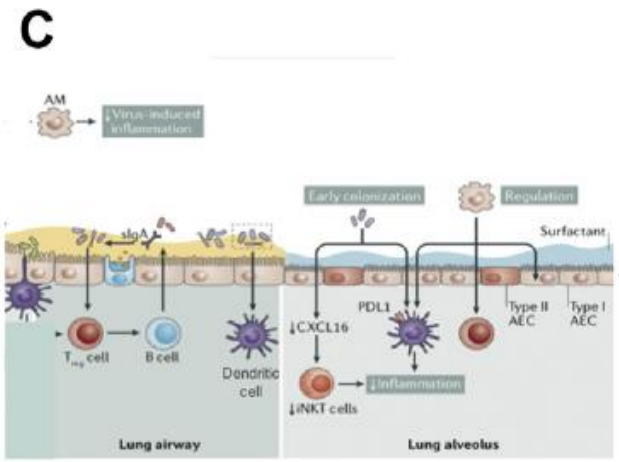
Van Splunter, Mucosal Immunology, 11 (4), 1254-1264, 2018

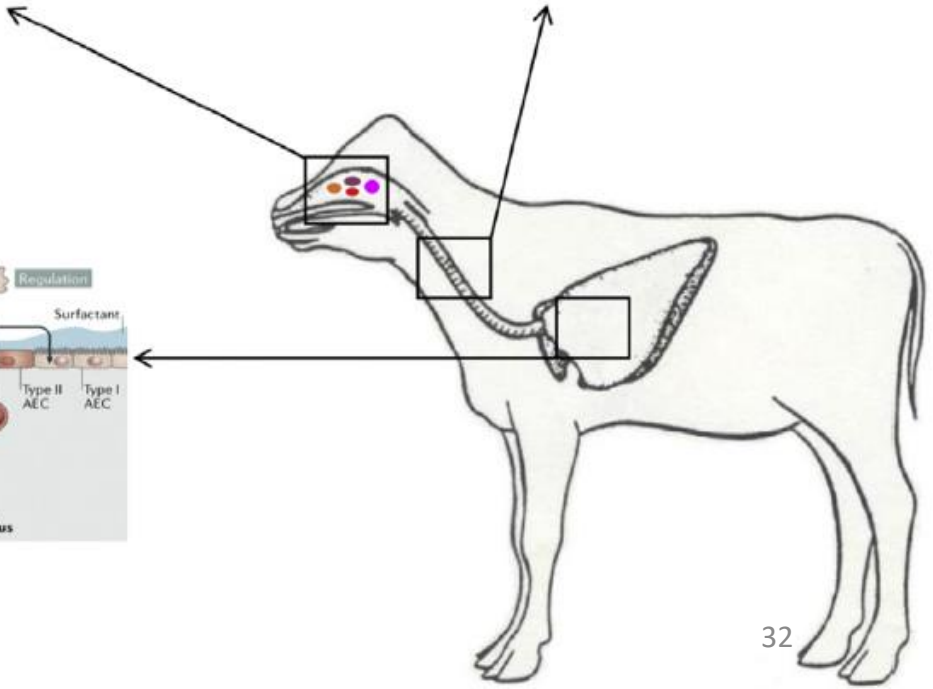
Respiratory mucosal immune system





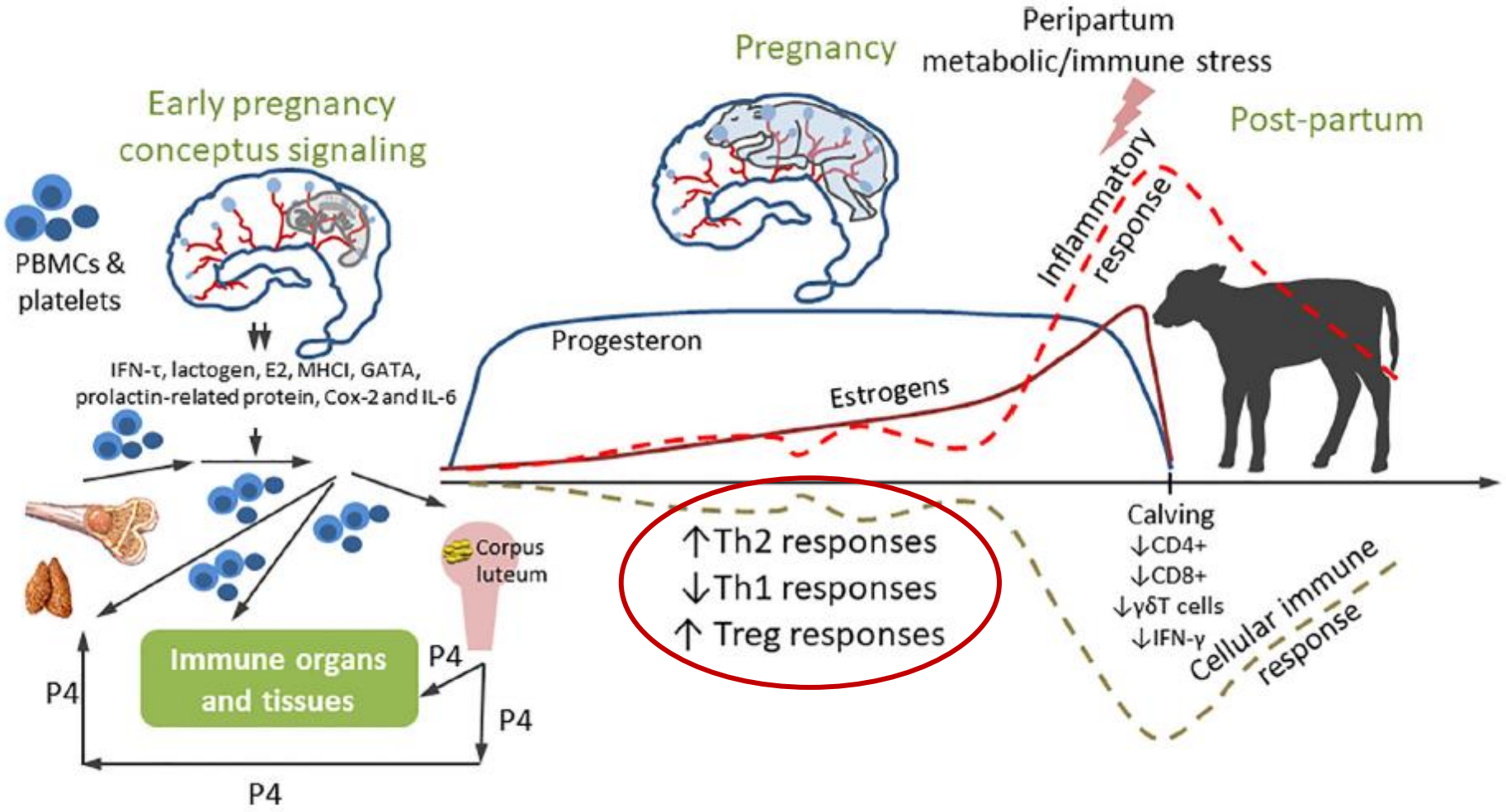






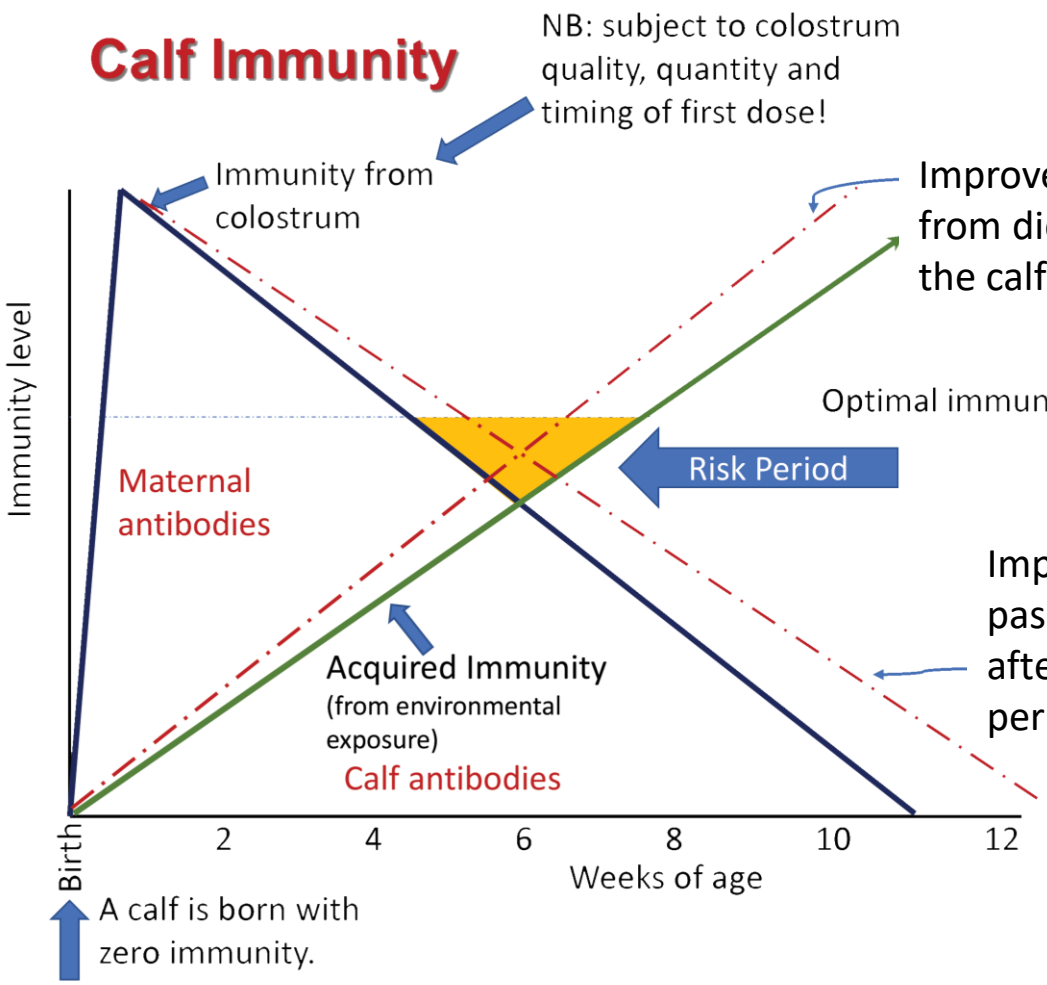
Chase and Kaushik.
Vet Clin Food Anim
35 (2019) 431–451

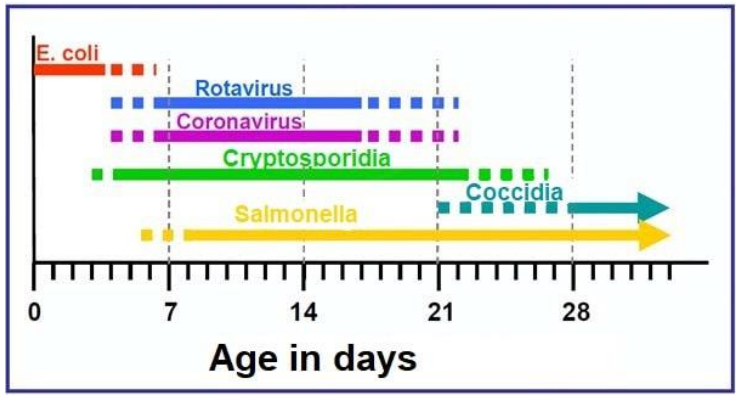
Immune modulation during pregnancy



Vlasova AN and Saif LJ (2021) Bovine Immunology: Implications for Dairy Cattle
Front. Immunol. 12:643206. doi: 10.3389/fimmu.2021.643206

The immunity gap

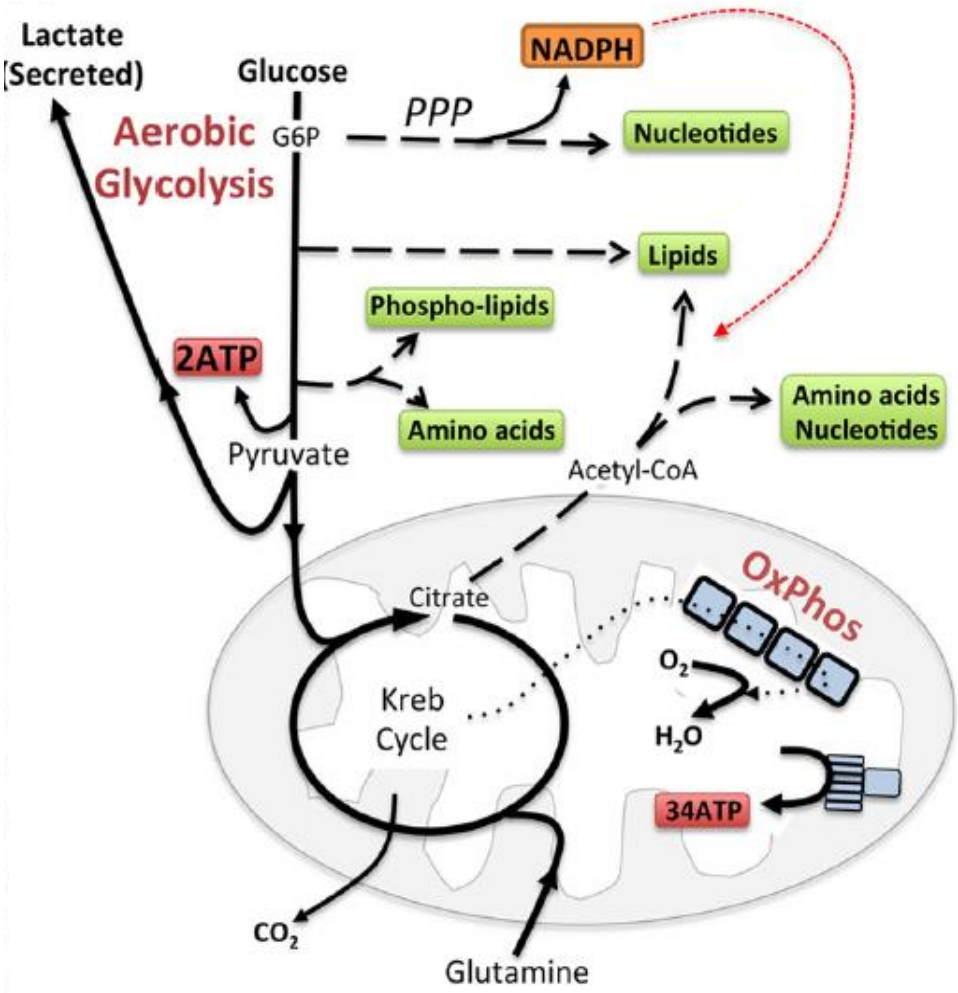




Common disease-causing pathogens in calves

Agriland Team, 2021

Molecular and translational basis of immunometabolism



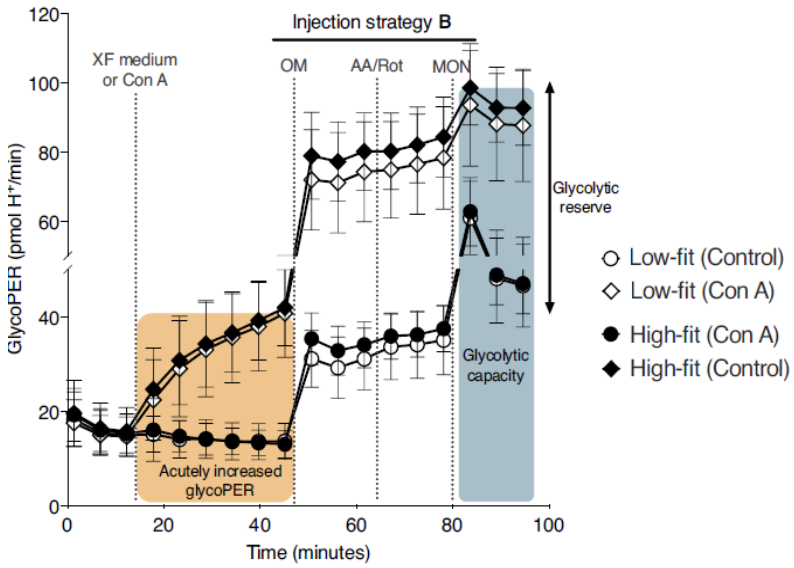
Larger glucose quantities
Faster production of small amounts of ATP
→ Increased glycolytic flux

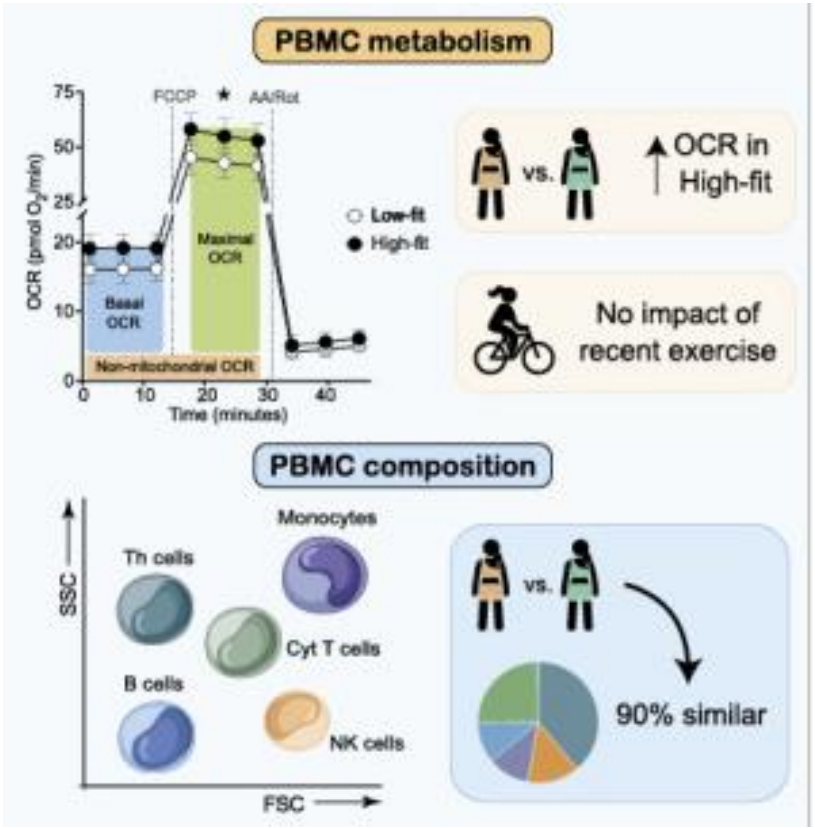
Norata et al. Immunity 43:421; 2015

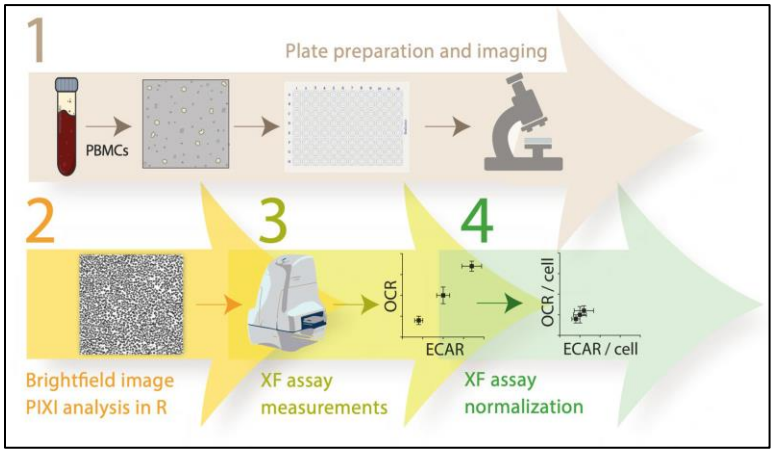
Extracellular flux analyses reveal differences in mitochondrial PBMC metabolism between high-fit and low-fit females

Am J Physiol Endocrinol Metab 322: E141; 2022

Joëlle J. E. Janssen,[1,2] Bart Lagerwaard,[1,3] Mojtaba Porbahaie,[2] Arie G. Nieuwenhuizen,[1] Huub F. J. Savelkoul,[2] R. J. Joost van Neerven,[2] Jaap Keijer,[1] and Vincent C. J. de Boer[1]







Janssen et al., Scientific Reports (2021) 11:1662

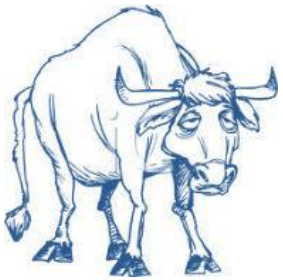
Identification new natural resistance biomarkers in milk

Proteomics approach on milk proteins by Nano LC-MS/MS

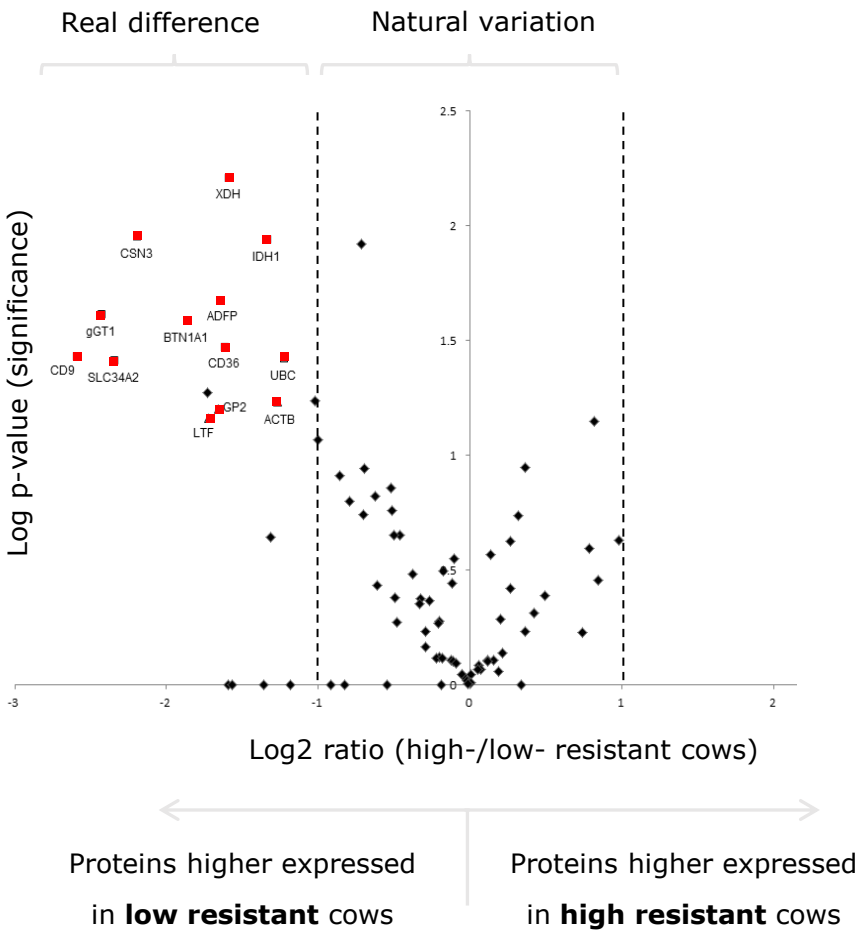
Selected high and low disease resistant cows

NanoLC-MS/MS

vs



- 78 proteins identified
- 13 higher in low-resistant cows
- Lactoferrin validated and associated with low-resistant cows, increased lameness, and prognostic marker for early culling



Van Altena et al., Veterinary Immunology and Immunopathology 174 (2016) 11–18

ISSN 1466-2523

Animal Health Research Reviews 13(1); 129–141
doi:10.1017/S1466252312000096

Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract

Ryan Taschuk[1,2] and Philip J Griebel[1,2]*

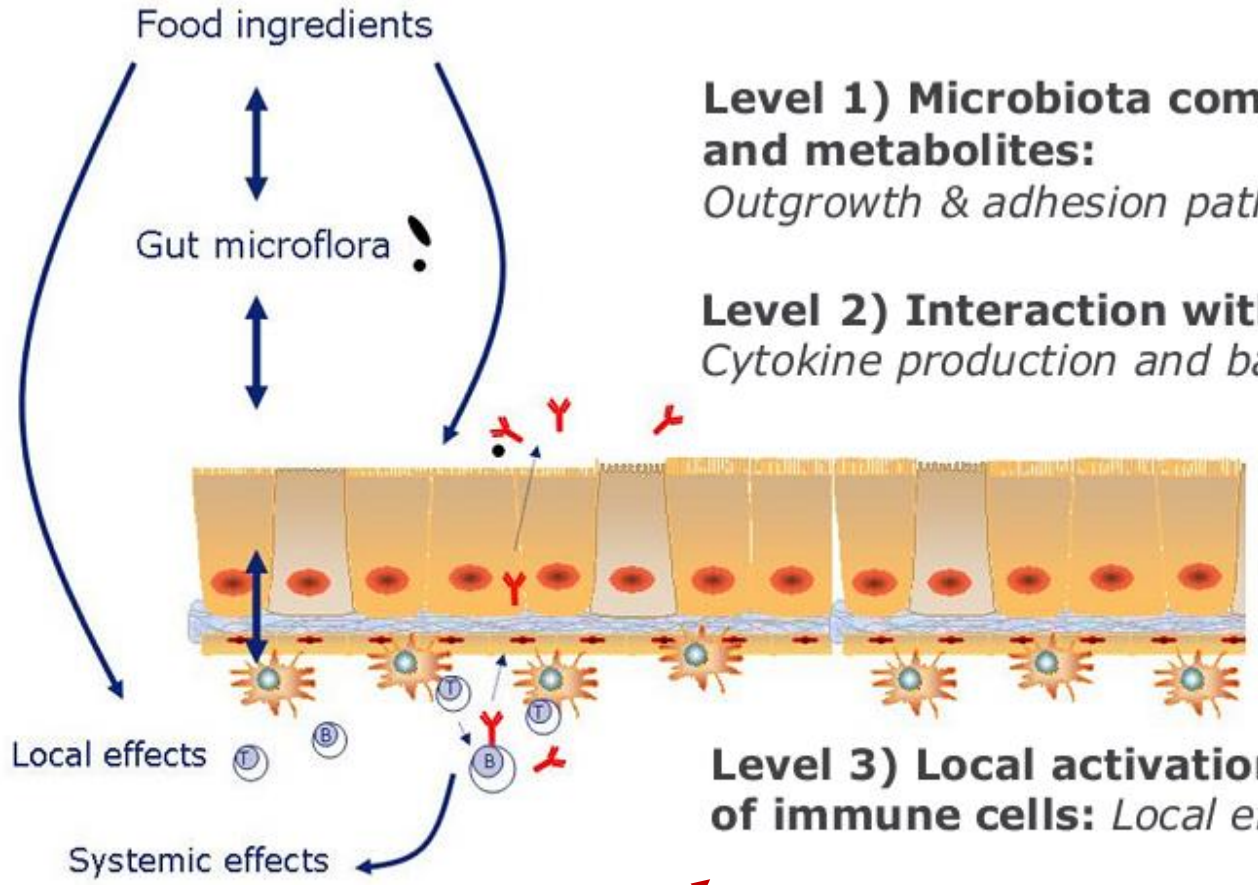
Increasing use of mucosal vaccines to enhance immunity in new-born calves and circumvent vaccine interference associated with passive transfer of maternal antibody

Induction of protective immune responses following oral or intranasal vaccination will be influenced by the state of mucosal immune system maturation and activation at the time of vaccination. The neonatal mucosal immune system is functional and responds to vaccination

Use of bacterial vectors for oral vaccine delivery might interfere with the microbiome and thus affect vector persistence and the amount of vaccine antigen expressed in the GIT

The activity of adjuvants incorporated into mucosal vaccines may be altered as the microbiome modulates TLR expression by the mucosal immune system

Food modulates gut immunity at four distinct levels



Level 1) Microbiota composition and metabolites:
Outgrowth & adhesion pathogens

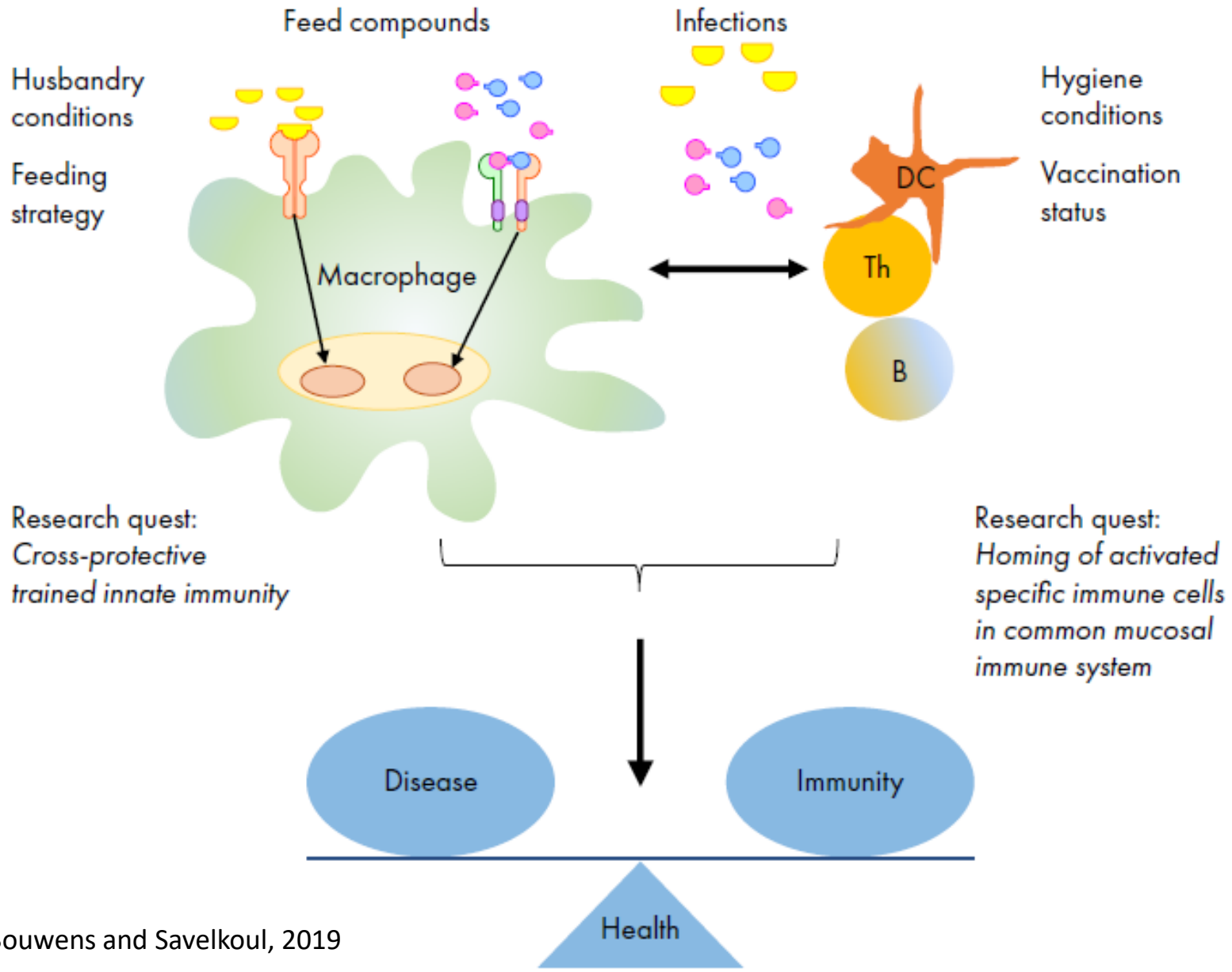
Level 2) Interaction with epithelia:
Cytokine production and barrier function

Level 3) Local activation of immune cells: *Local effects*

Level 4) Migration of immune cells or uptake in blood :
Systemic/distant effects

Modulating factors

Current perspectives on protective immunity



Bouwens and Savelkoul, 2019

Conclusions

• Mucosal tolerance is important for elimination of pathogens in the absence of an epithelium-damaging inflammatory response.
• Dendritic cells regulate tolerance or immune activation by the induction and expansion of regulatory T cells.
• Homing of gut-activated immune cells can occur within the common mucosal immune system towards the upper airways, thereby, providing protection against infections.
• Nutritional compounds can exert immunomodulatory activity by influencing mucosal macrophages and dendritic cells.
• Nutritional compounds can exert innate immune training and thereby induce enhanced innate immune responses and cross-reactive activity.
• Priming of the immune system in the mother to enhanced immune activity can be (epigenetically) transferred to the offspring providing these with better immune protection.
• Immunomodulation by dietary components is a feasible option to steer immune competence and improved resistance.