THE GUT MICROBIOME IN TUBERCULOSIS

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- The Gut microbiota and their importance
- *Mycobacterium tuberculosis* summary
- Microbiota and tuberculosis
- Conclusion

Front. Nutr. 8:626254.

Microbiome

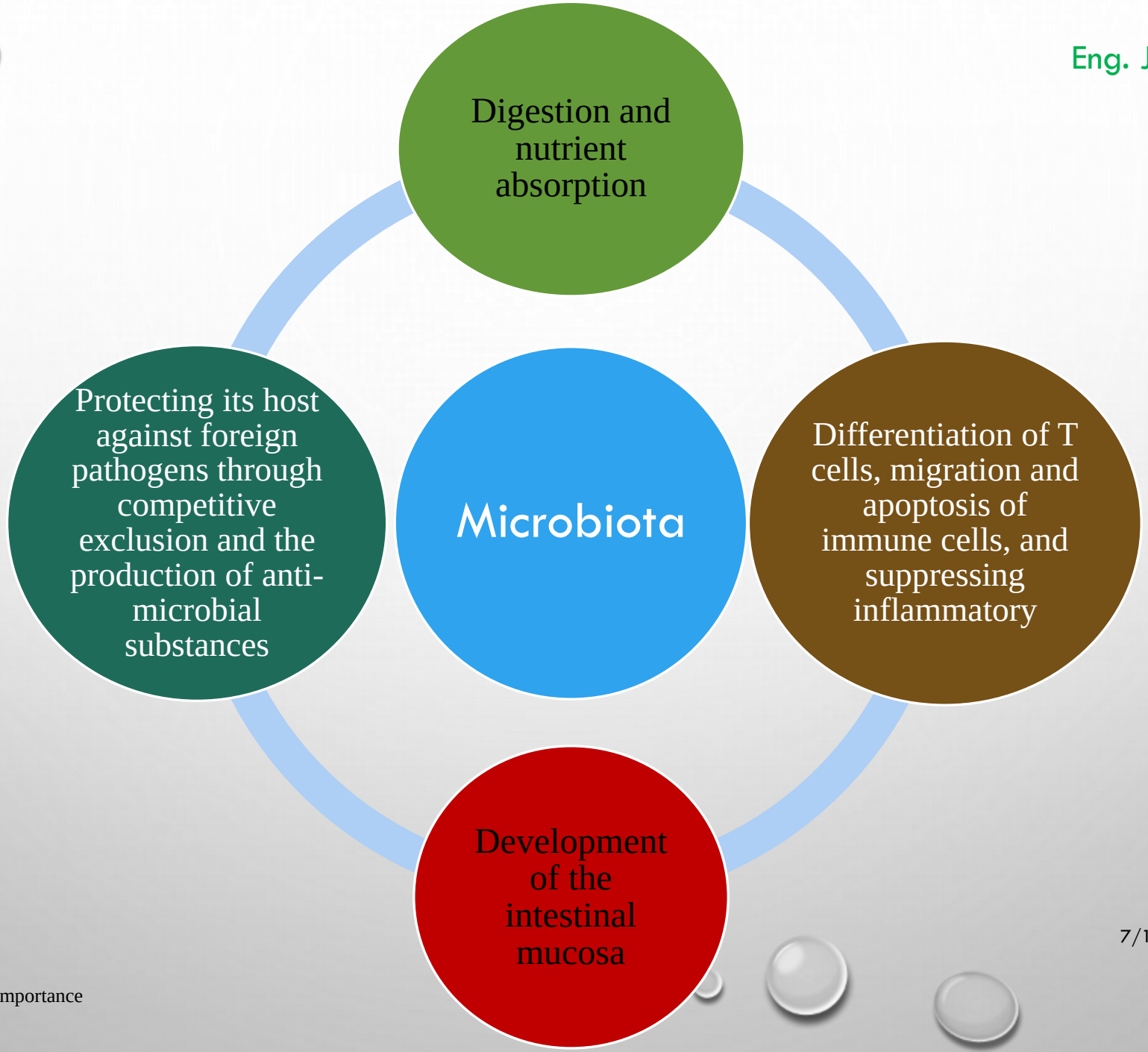
- Collective genomes of the micro-organisms in a particular environment

Microbiota

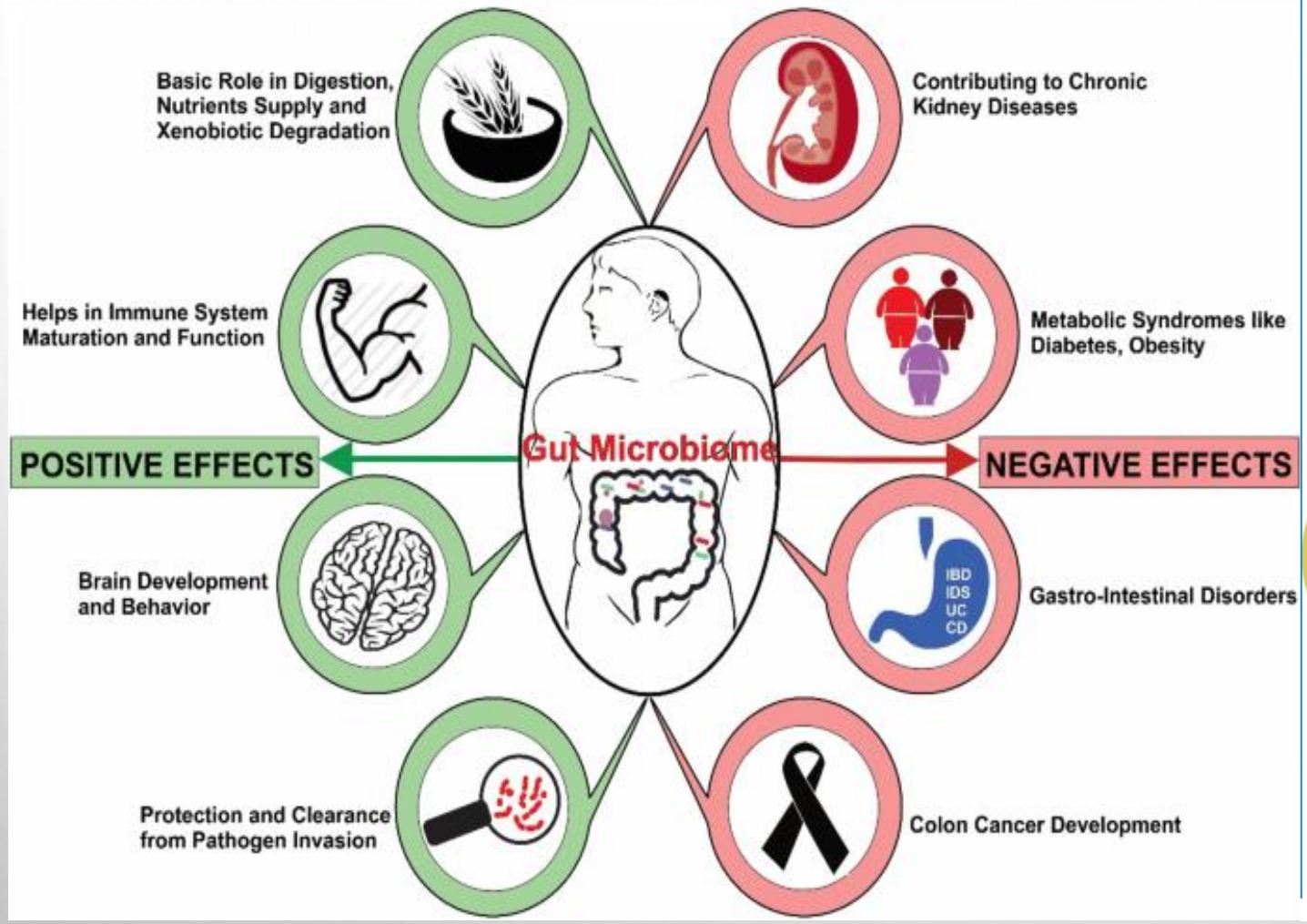
- Community of micro-organisms themselves

- The human genome consists of about 23 000 genes, whereas the microbiome encodes over three million genes producing thousands of metabolites, which replace many of the functions of the host, consequently influencing the host's fitness and health.
- Accounting for 1–3% of our total body weight

Eng. J 3 (2017) 71–82



Arch Microbiol (2020) 202:2147–2167



Eng. J 3 (2017) 71–82

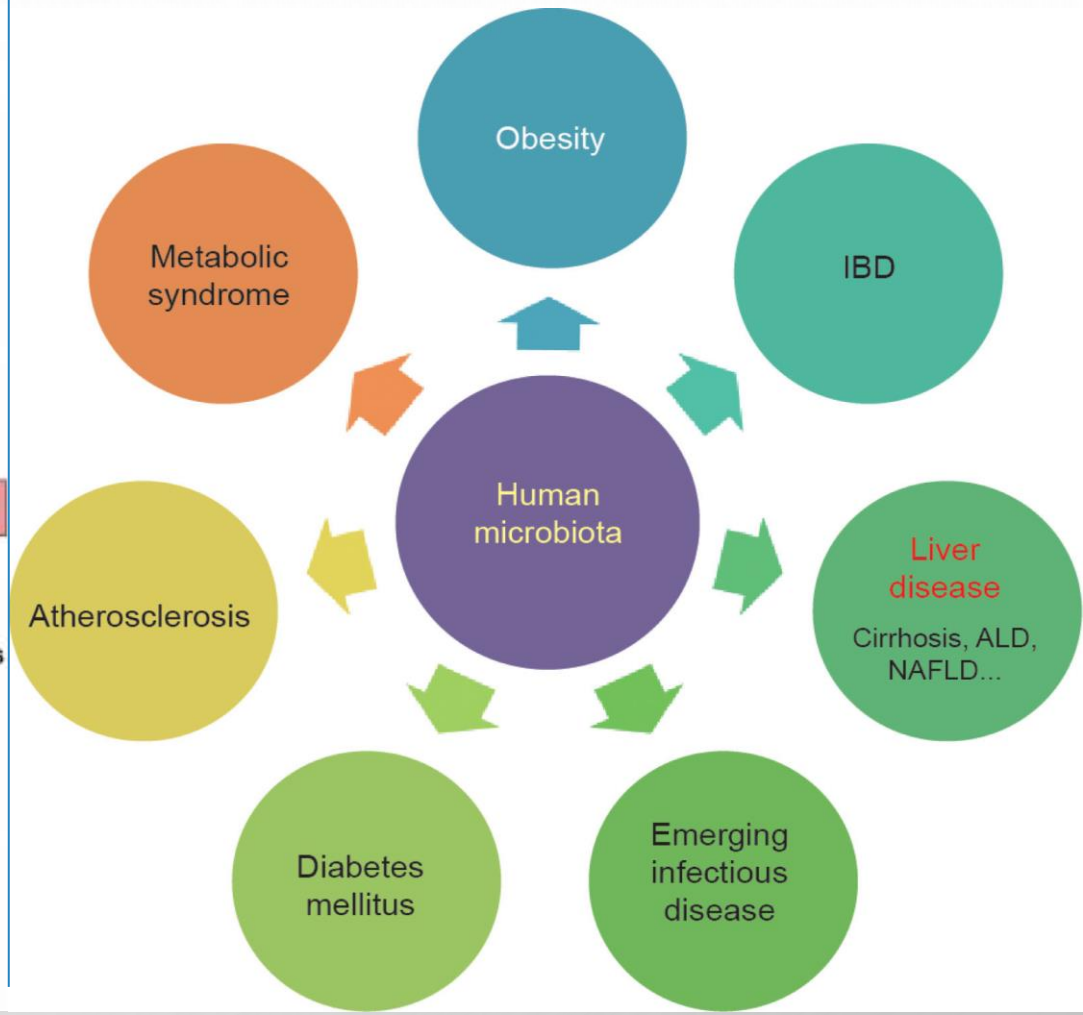


Fig. 2 The link between the gut microbiome and its impact over the host-associated health and disease attributes

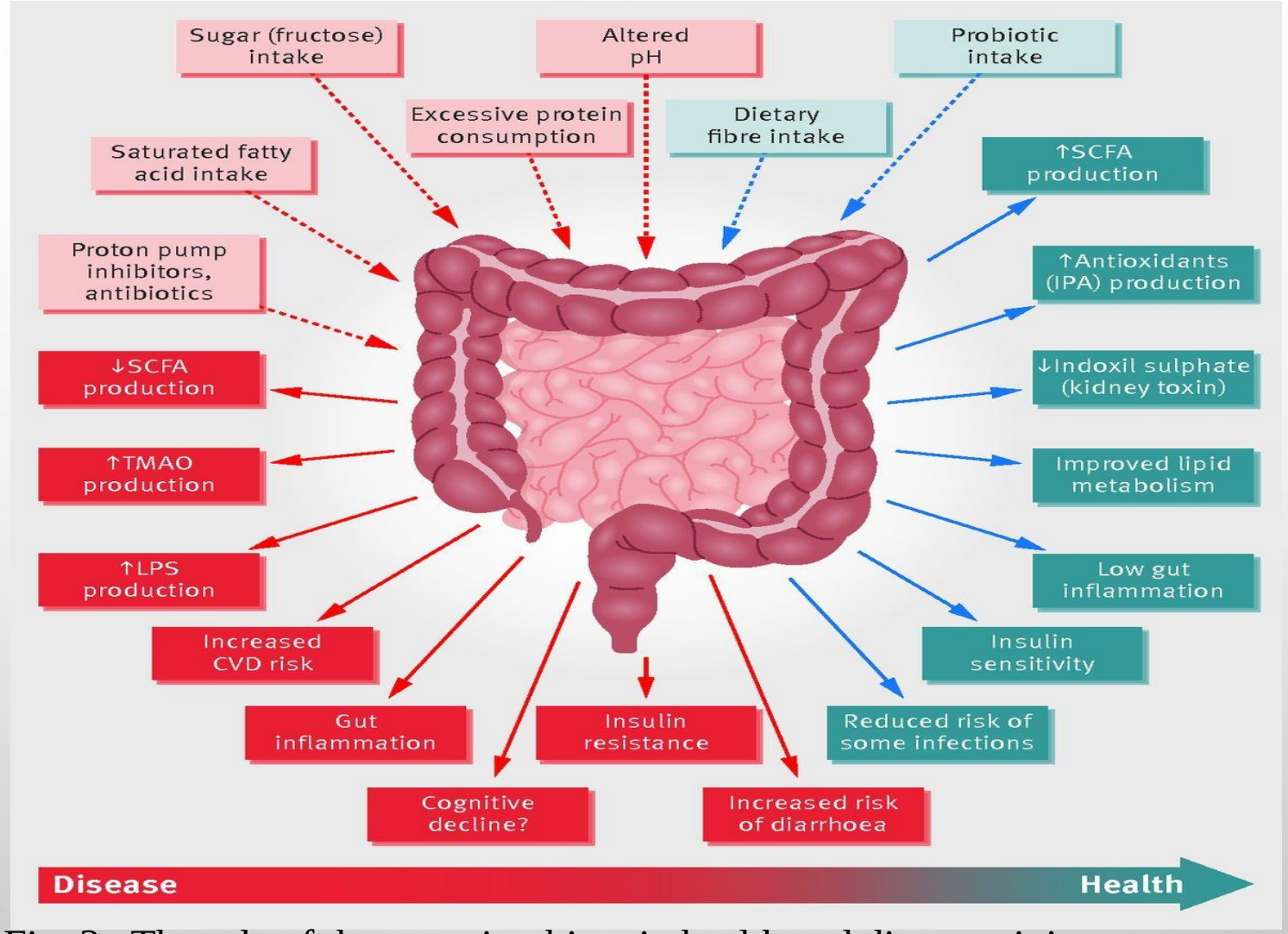
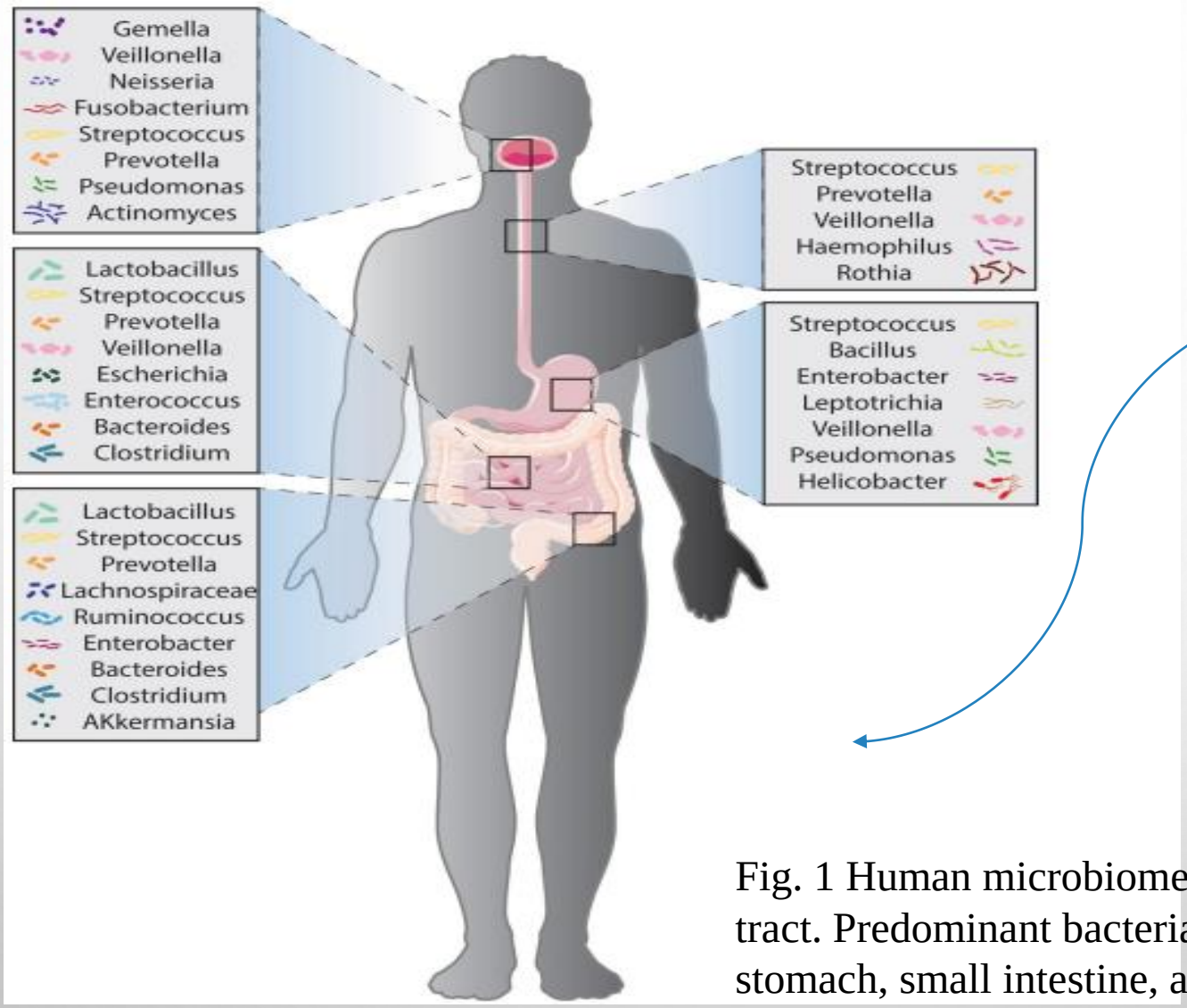


Fig. 3 The role of the gut microbiota in health and disease giving some examples of inputs and outputs.
CVD=cardiovascular disease; IPA=indole propionic acid; LPS=lipopolysaccharide; SCFA=short chain fatty acids; TMAO=trimethylamine N-oxide

Dig. Dis. Sci. 2020 Mar;65:695-705



Microbiota of the human GI tract contains bacterial (microbiota), viral (virome), and fungal (mycobiota) species

Fig. 1 Human microbiome composition varies by location in the GI tract. Predominant bacterial genera in the oral cavity, esophagus, stomach, small intestine, and colon are delineated in this figure

Dig. Dis. Sci. 2020 Mar;65:695-705

The Gut microbiota and their importance

- The colonization of the host by micro-organisms begins within minutes of birth.
- Alteration in gut microbiota (dysbiosis), resulting in immunological dysregulation, is associated with the development of chronic respiratory diseases, such as allergy, asthma, COPD, and cystic fibrosis

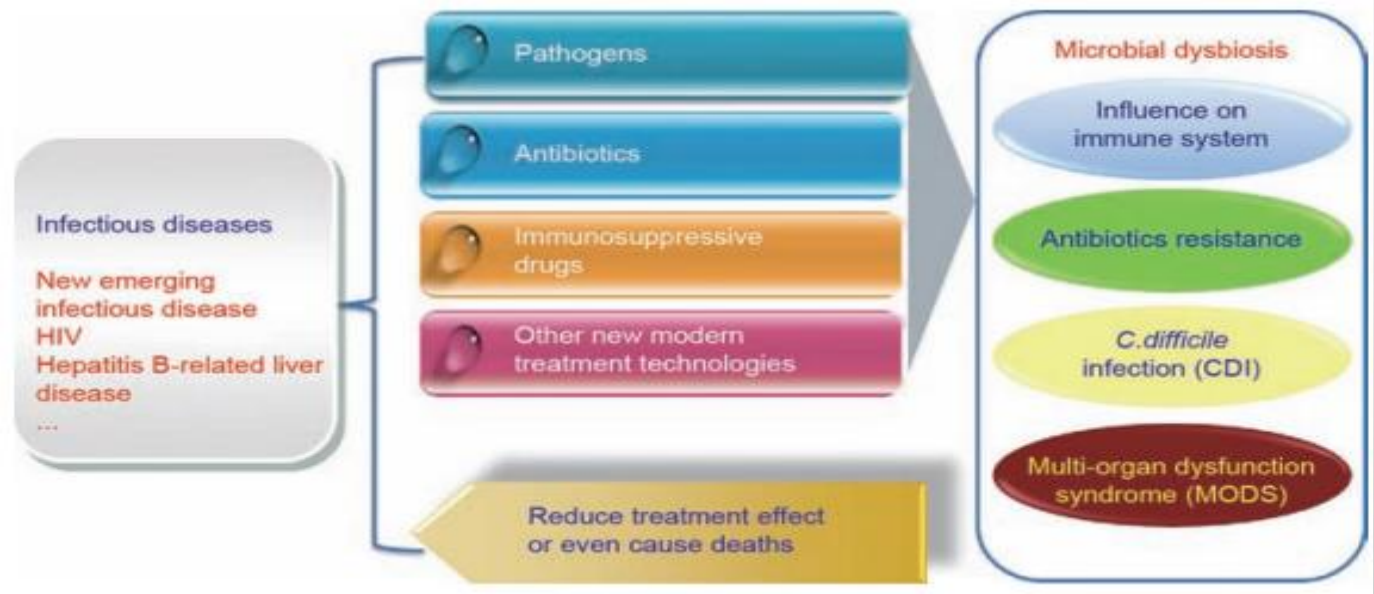


Fig. 4 Infectious diseases have a profound impact on the human microbiota. The wide use of antibiotics, immunosuppressive drugs, and other new treatment technologies for infectious diseases such as frequently emerging infectious diseases, HIV infection, and CDI has a profound impact on the human microbiota, which in turn determines the outcome of the infectious disease in the human host.

Eng. J 3 (2017) 71–82.

- The normal gut microbiome produces 50–100 mmol·L-1 per day of short-chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids, and serves as an energy source to the host intestinal epithelium.
- These SCFAs can serve many roles in:

1. Regulating gut motility
2. Inflammation
3. Glucose homeostasis
4. Energy harvesting
5. Deliver vitamins to the host, such as folates, vitamin K, biotin, riboflavin (B2), cobalamin (B12)
6. Stimulate the development of the humoral and cellular immune system

- Sweeteners, like sucralose, aspartame, and saccharin or food additives such as carboxy-methyl-cellulose and polysorbate-80 have been shown to disrupt the balance and diversity of gut.

Dig. Dis. Sci. 2020 Mar;65:695-705

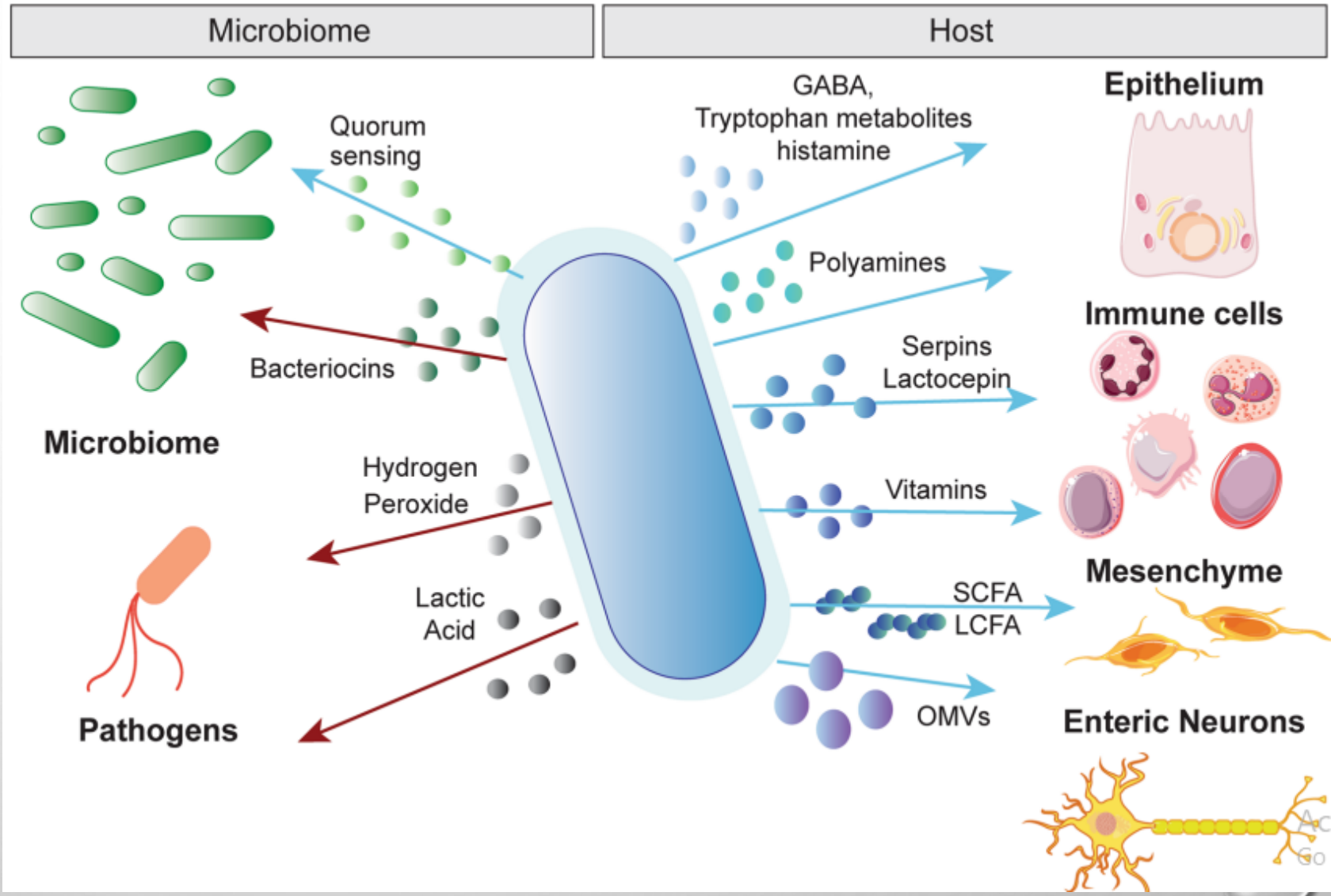


Fig. 5 Microbial metabolites produced in the GI tract have diverse functions. The GI microbiome can modulate both intra- (microbe–microbe) and inter-kingdom (microbe-host) interactions that can influence human health. Bacteria are involved in quorum sensing and can release bacteriocins, hydrogen peroxide, and lactic acid, which yield efects on the gut microbiome and pathogens. In addition, bacteria can produce gamma-aminobutyric acid (GABA), tryptophan metabolites, histamine, polyamines, serpins, lactocepin, vitamins, short chain fatty acids (SCFA), long chain fatty acids (LCFA), and outer membrane vesicles (OMVs), which can have efects on the human host epithelium, immune cells, mesenchyme, and enteric neurons

GUT MICROBIOTA CALIBRATES THE HOST IMMUNITY BY:

1. Influencing the release of pro-inflammatory (IFN-γ, IL-17, IL-6, and IL-12) and anti-inflammatory IL-10 cytokines
2. Metabolites release
3. Controlling function of mononuclear phagocytes including dendritic cells (DCs)
4. MiRNAs

Gut microbiota modulates host immune response at extra-intestinal sites such as the brain, bone marrow and lung

- Metabolites produced by gut bacteria may travel along the mesenteric lymphatic system to the circulatory system and subsequently enter pulmonary circulation, which may lead to the local activation of immune responses.

- IFN-γ producing CD4 + T cells provide the major effector response to TB and IFNγ is required for protection against disease progression in TB.

Cell. Mol. Life. Sci (2020) 77:1497–1509

SCFAs

- Important role in inflammatory responses in the gut and at distal mucosal sites such as the respiratory tract.

- Many cells express G protein coupled receptors (GPCRs) such as GPR41, GPR43 and GPR109A, and SCFAs activate host immunity by interacting with these receptors.

- SCFA can induce pro- or anti-inflammatory responses depending on the signal transduction pathway.

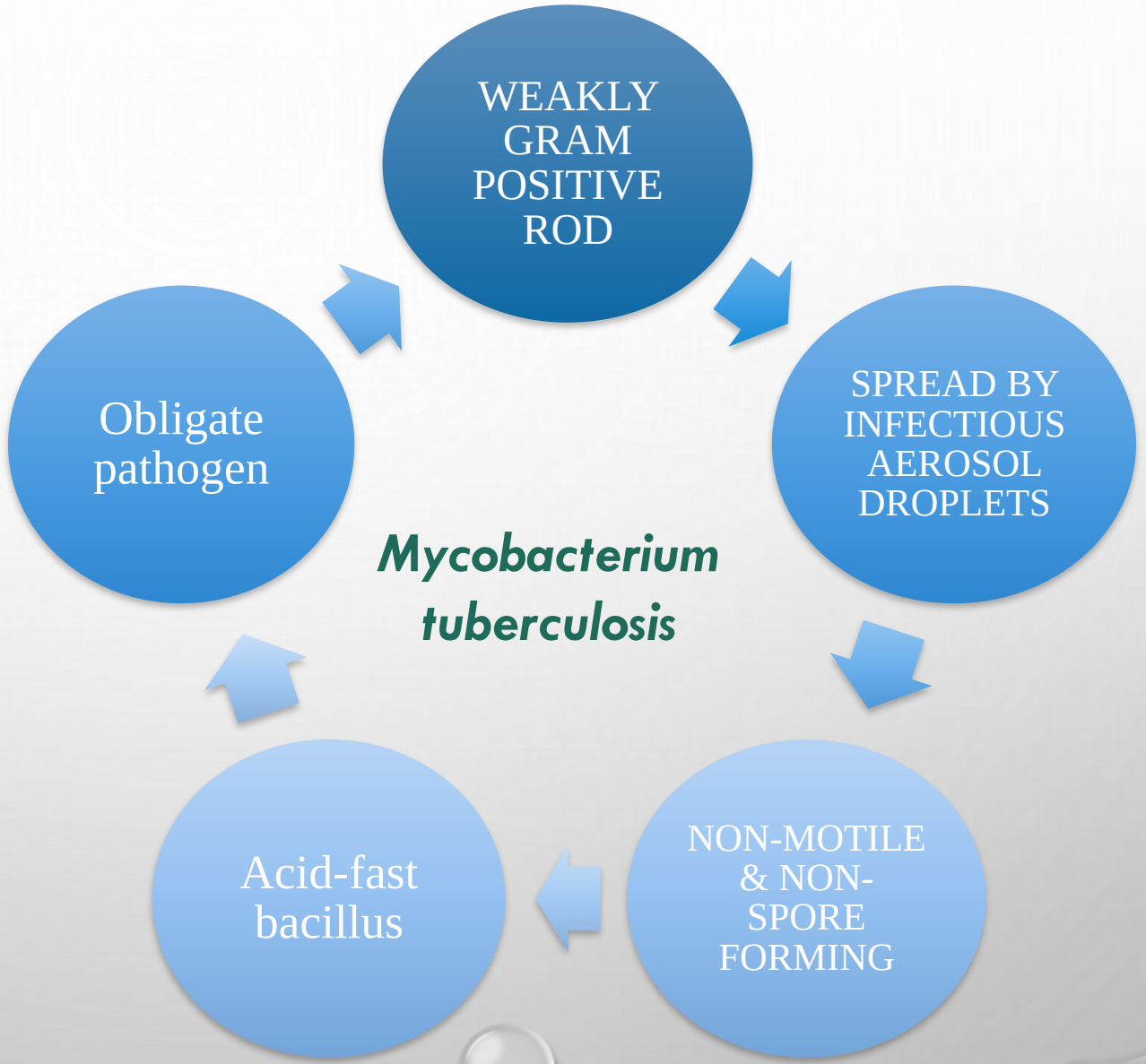
→ GPR41 and GPR43 signaling can commit to mitogen-activated protein kinases (MAPK) activation thereby inducing a pro-inflammatory response.

→ GPR43 can activate β-arrestin-2 activation pathway resulting in an anti-inflammatory milieu through the inhibition of NF- κB.

- SCFAs that induces both pro- and anti-inflammatory responses.
- ⟶ Butyrate stimulates the secretion of IL-10 from dendritic cells and macrophages in the gut by signaling through GPR109A.
- SCFAs also promote the expansion of Treg particularly along the gut–lung axis through the inhibition of histone deacetylase.

Increase in systemic inflammation and concomitant impairment of immune responses in TB patients may imply loss of microbiota that produce SCFAs

- Type 2 diabetes (T2D) is associated with a decrease in the abundance of SCFA producers.
- T2D poses a significantly increased risk for the development of active TB.


WEAKLY GRAM POSITIVE ROD
SPREAD BY INFECTIOUS AEROSOL DROPLETS
NON-MOTILE & NON-SPORE FORMING
Acid-fast bacillus
Obligate pathogen
Mycobacterium tuberculosis

FACTS ABOUT TB

1. Second most common cause of death in humans by an infectious agent after HIV/AIDS (estimated 1.4 million deaths annually)

2. Morbidity and mortality are associated with active TB disease, 5% to 10% of individuals that are exposed to and infected by *M. tuberculosis* showing symptoms, such as bad cough, fever, weight loss, chest pain, and night sweats

3. One-fourth of the world's population has latent *Mtb* infection with no clinical symptoms

4. Age, immune deficiencies, malnutrition, and bacterial load are the most important factors for the rapid replication of *M. tuberculosis* and progression to active TB

Mycobacterium tuberculosis summary

mBio. 2018 Sep 18;9(5)

BMJ Open 2022

❖ One emerging host factor that may be associated with TB disease is the gut microbiota[16]

FACTS ABOUT TB

5

- Diagnosis is largely based on immunological tests (e.g., PPD; IFNγ release assays (IGRA).

6

- A range of innate and adaptive immune mechanisms governed by genetic and epigenetic factors.

7

- First-line anti-TB antibiotics isoniazid, pyrazinamide, and ethambutol are narrow-spectrum, showing little or no activity outside the mycobacterial genus, but are often combined with the broad-spectrum antibiotic rifampin, which affects a wide range of Gram-positive and Gram-negative bacteria.

8

- One of the longest duration antibiotic regimens. Given the recognized effects that antibiotics have on the composition and function of the host microbiome

PLoS Pathog (2021) 17(4):e1009377

FACTS ABOUT TB

9
- Increasing outbreaks of drug-resistant TB and TB/HIV co-infection pose a significant threat to treating and preventing further transmission

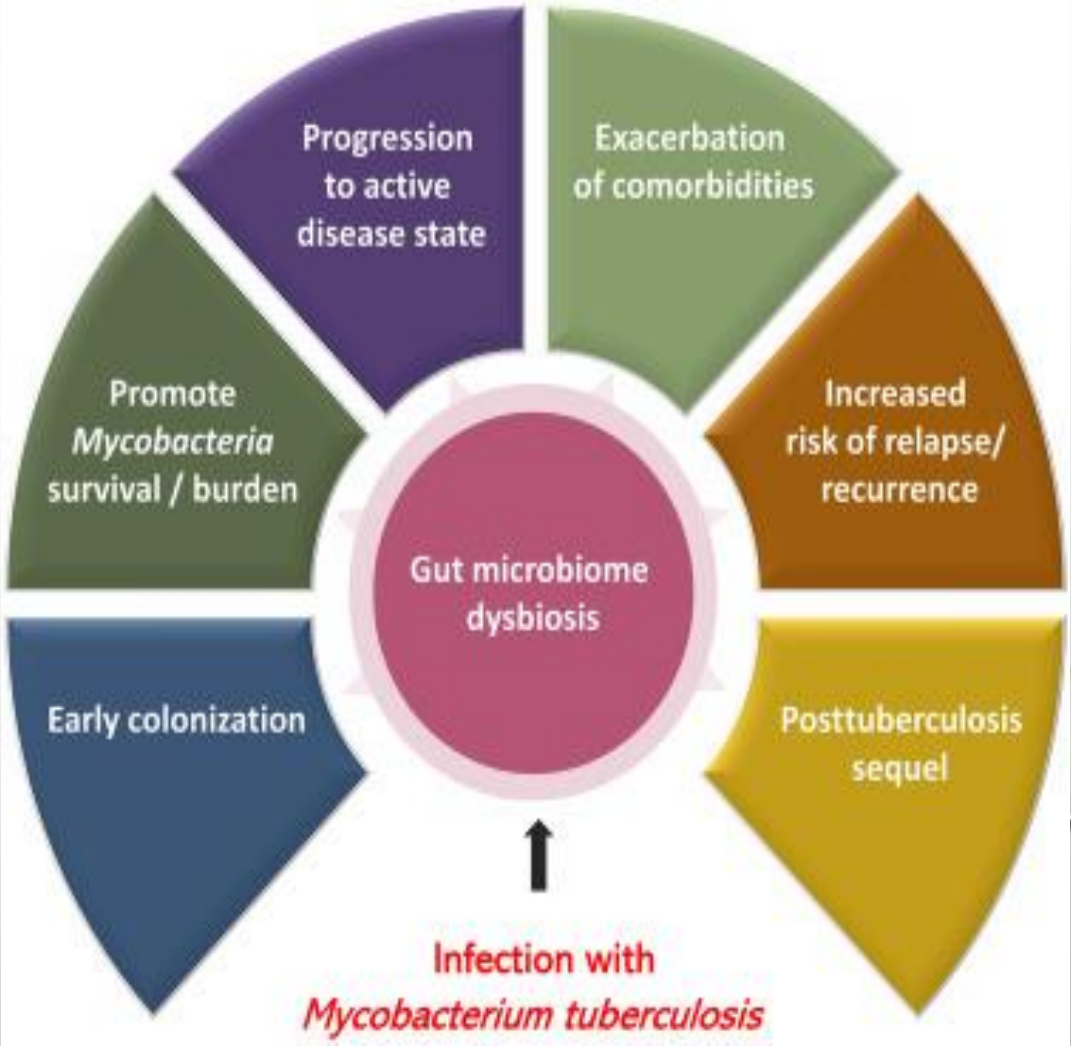
10
- *Mtb* has the ability to evade the immune system over prolonged time periods, even decades after exposure, in the form of latent TB

11
- Risk factors for developing active TB, including HIV, malnutrition, smoking, alcohol, and diabetes, are associated with both structural and functional changes in the gut microbiota

12
- Long-lasting impact on the microbiota is likely to have deleterious consequences for susceptibility and immune control of infectious diseases, including TB

Front. Immunol. 9:709.

PLoS Pathog (2021) 17(4):e1009377



mBio. 2018 Sep 18;9(5)

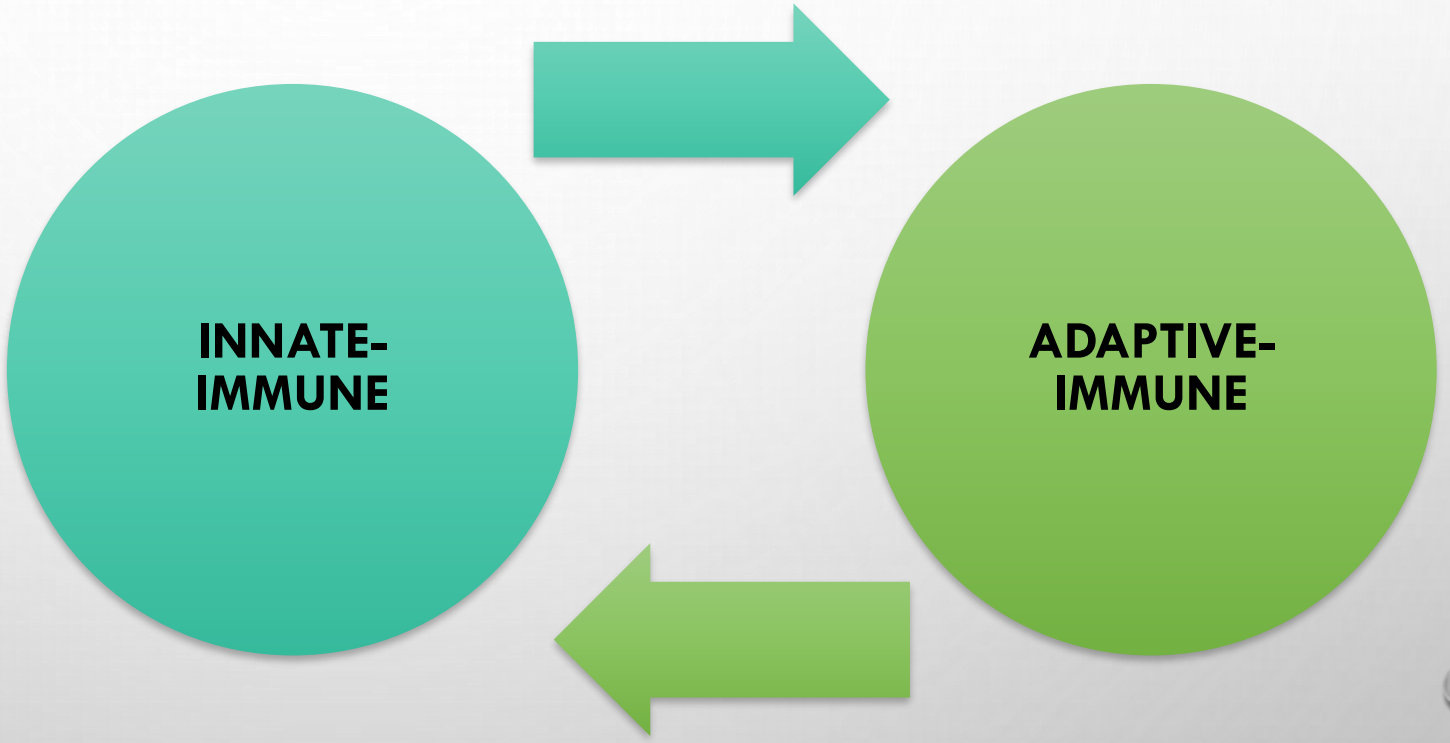
Relationship between gut dysbiosis and tuberculosis

THE MICROBIOME MIGHT CONTRIBUTE TO TUBERCULOSIS RISK AND DISEASE

- Disrupt in immune cell subsets or function that may influence tuberculosis susceptibility or response to therapy
- Gut microbiota regulates mincle mediated activation of lung DCs to protect against *Mtb*
- Affecting drug absorption during tuberculosis treatment
- Down-regulated of LncRNA-CGB

M. tuberculosis infection is extremely peculiar due to the unique virulence factors of this bacteria and the immune response triggered by this infection. Both innate and adaptive immunity play a crucial role in controlling *M. tuberculosis* infection, and their interaction contributes to the clinical manifestations of the disease. *M. tuberculosis* uses multiple immune evasion mechanisms, which can prevent the formation of an immune response capable of eradicating the infection.

Disrupt in immune cell subsets or function that may influence tuberculosis susceptibility or response to therapy



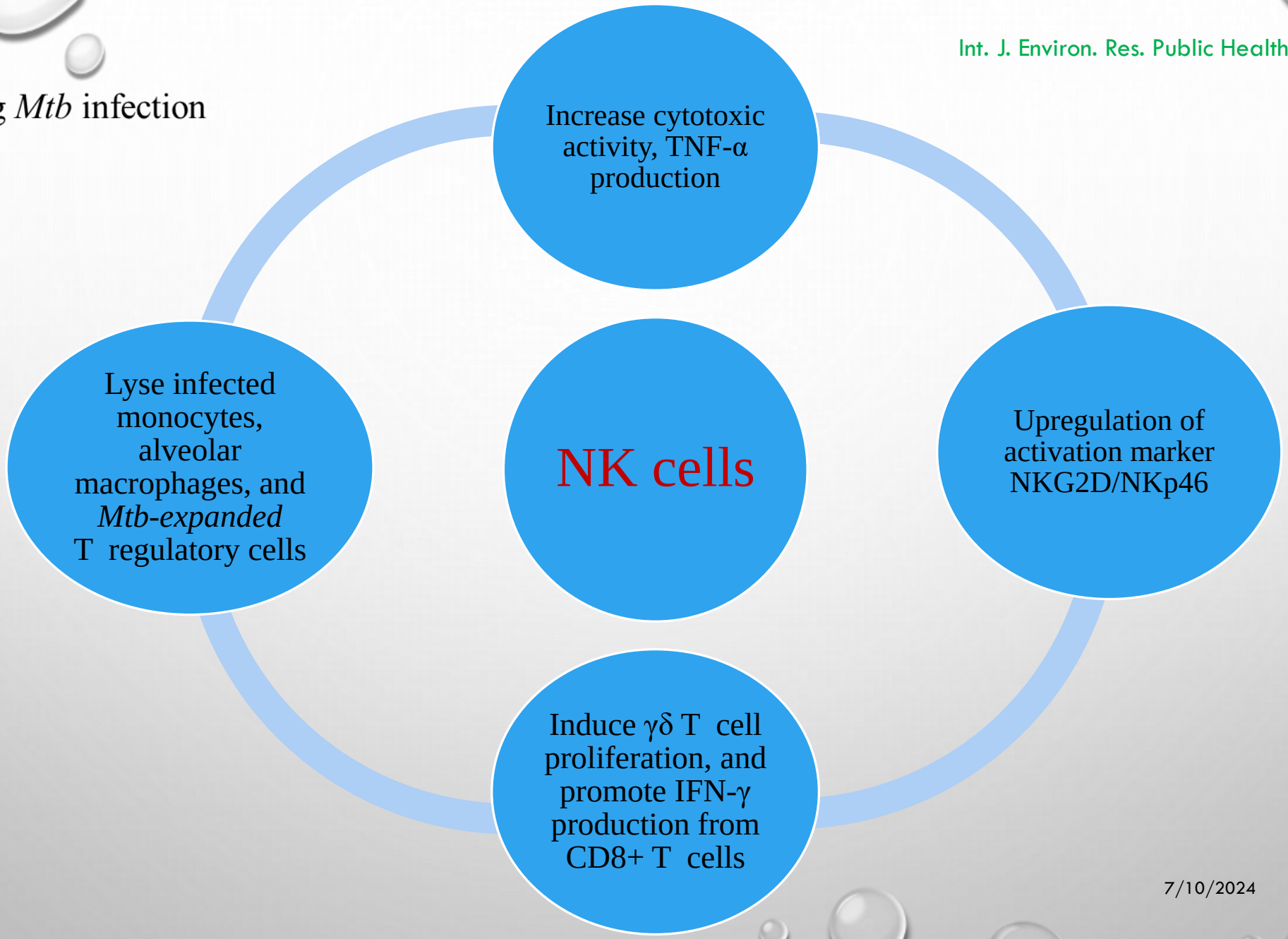
Innate immune

- ✓ The immune response to *M. tuberculosis* is activated by the exposure of the airway epithelium to the bacillus.
- ✓ After exposure, *M. tuberculosis* can infect the cells of the respiratory mucosa ⟶ stimulate the production of some cytokines, in particular interferon-gamma (IFN-γ).
- ✓ TST-negative individuals demonstrated lower TNF-α induction in response to LPS stimulation compared to TST-positive people. These results clearly demonstrated that measurement of a single parameter such as TNF-α is not sufficient.

- The ability to eradicate *M. tuberculosis* depends on the microbicide functionality of the alveolar macrophages and the virulence factors of the mycobacteria.

Int. J. Environ. Res. Public Health 2021, 18,12220

During *Mtb* infection

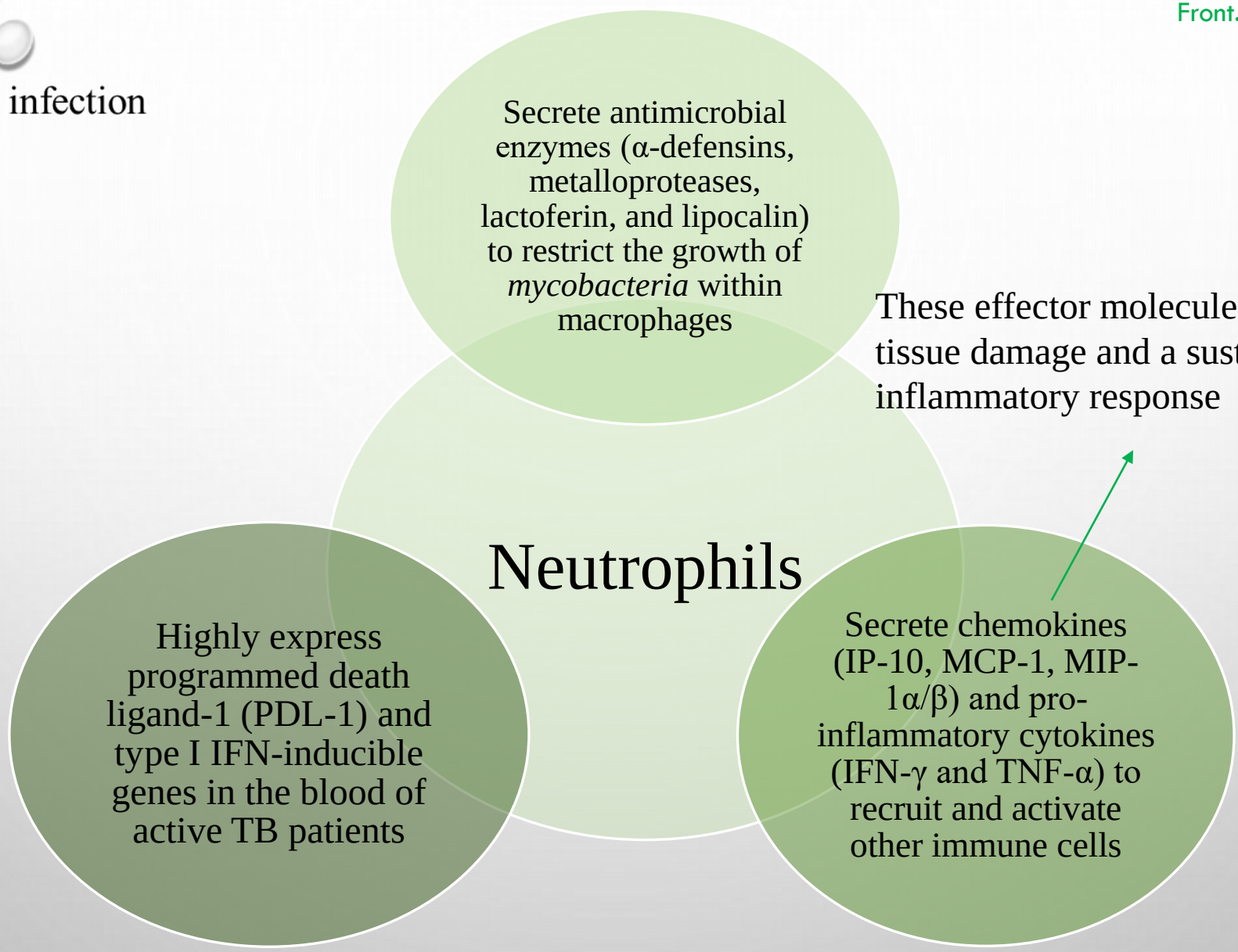


- γδ T cells are a distinct subset of CD3+ T cells. Recognize unprocessed, non-peptide phosphate antigens in a non-MHC restricted manner. γδ T cells represent an early defense against pulmonary TB and serve as a link between innate and adaptive immunity.

- During the initial phase of *Mtb* infection, γδ T cells are recruited in the lungs, which express IFN-γ and IL-17 along with cytotoxic effector function.

- Increased frequency of γδ T cells has been shown in lungs in patients with active TB.

During *Mtb* infection



These effector molecules also mediate lung tissue damage and a sustained, hyper-activated inflammatory response

Innate lymphoid cells (ILCs) are derived from lymphoid cell progenitor cell at mucosal surfaces, and play a significant role as a first line of defense against pathogens

Three groups of ILCs (ILC1, ILC2, and ILC3) have been defined based on shared expression of surface markers

Promote the barrier function of lung epithelium and lung tissue homeostasis in multiple chronic infectious and inflammatory diseases of the respiratory tract

During *Mtb* infection

Treatment of drug-susceptible TB was reported to restore the levels of ILC1 and ILC3 but not ILC2

ILC populations isolated from lungs of TB-infected individuals expressed high levels of activation markers CD69, CD25, and CCR6 compared to NK and T cells

ILCs

ILCs also interact with the microbiota and the mucosal epithelium that induces active adaptive immunity, and thus could shape the success or failure of a pathogen such as *Mtb* in establishing an active long-term infection

Innate immune

- ✓ *M. tuberculosis* has developed several mechanisms mediated by glycolipids and proteins of the bacterial cell wall (e.g., inhibition of the Ca2+/calmodulin pathway), preventing the cytotoxic activity of alveolar macrophages.

- ✓ Allows to survive and multiply within macrophages, leading to macrophage lysis and the release of cytokines and bacterial antigens into the extracellular environment.

The dendritic cells then migrate to the lymph nodes to present the antigens of *M. tuberculosis* to the T-naive lymphocytes and activate the adaptive immune response.

In conclusion

1. TLRs regulate the abundance and composition of the commensal intestinal microbiota, and maintain the integrity of tissues and mucous barriers.

2. NLRs contribute to modulating the composition of the intestinal microbiota.

- The NLRP6, together with microbial metabolites, it regulates the secretion of IL-18 and antimicrobial peptides by epithelial cells, the mucosal secretion of goblet cells, and is crucial in response to bacteria and viruses
- NOD-1 receptor acts as an innate sensor for the formation of adaptive lymphoid tissue and the maintenance of intestinal immune tolerance to commensal micro-organisms.
- The NOD-2 receptor prevents inflammation of the small intestine by limiting the growth of the *Bacteroides vulgatus*.

3. Neutrophil
4. NK cell
5. ILC

Cell. Mol. Life. Sci (2020) 77:1497–1509

Adaptive immune

- ✓ The adaptive response mediated by T-lymphocytes develops approximately 2–4 weeks after mucosal exposure to *M. tuberculosis*.

- ✓ Intestinal dysbiosis conditions are also associated with alterations in the mucosal adaptive immune response, both humoral and cell-mediated types.

Examples of microbiota regulation of the adaptive immune response

1. Secretory Ig-A, which plays a crucial role in protecting the mucosal barriers.

The relationship between microbiota and secretory IgA production is mutualistic:

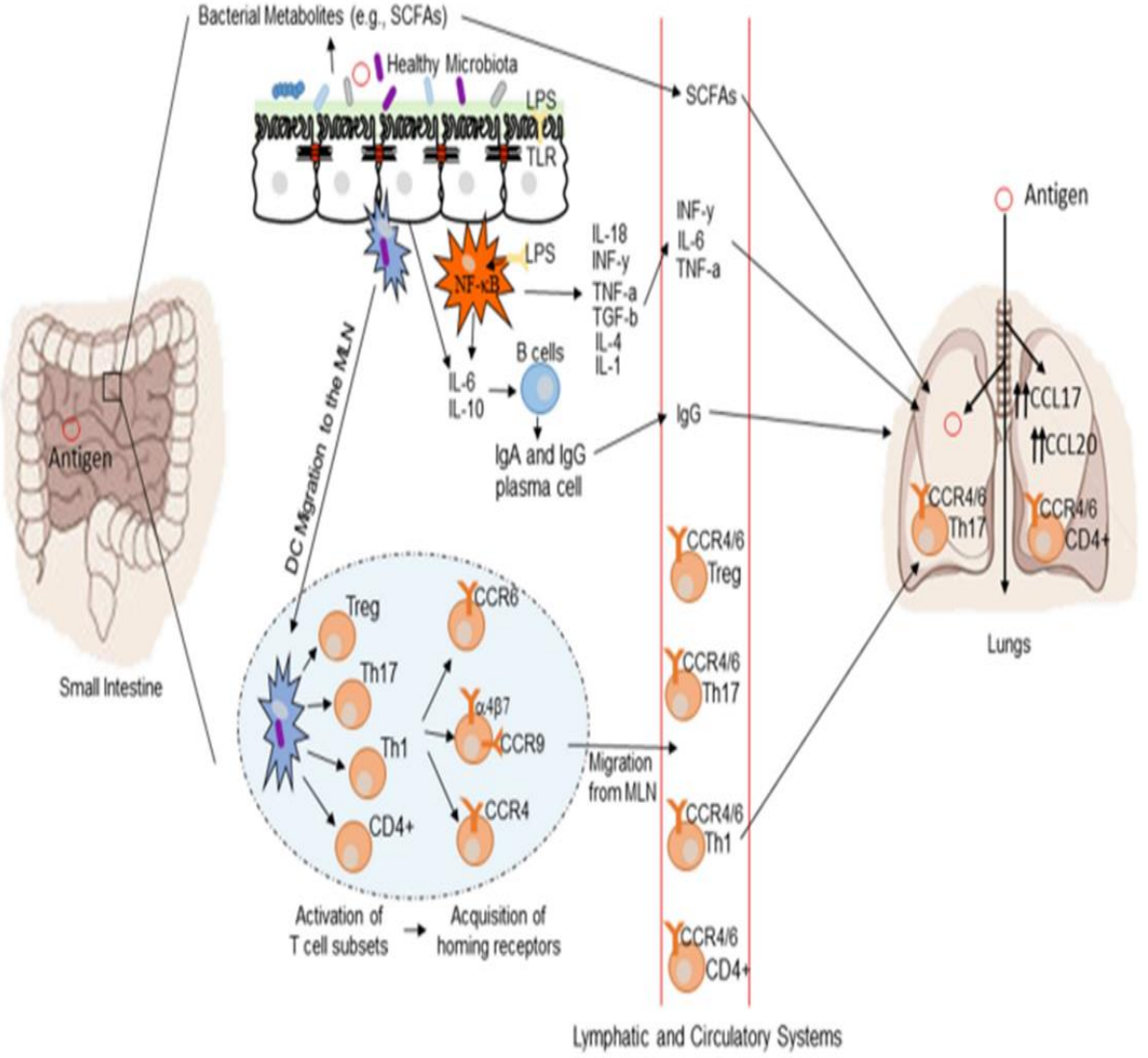
- ✓ The secretory IgA pool contributes to the maintenance of a specific type of commensal microbiome, also avoiding its excessive growth.
- ✓ The presence of specific bacterial species contributes to the production of this IgA family, stimulating the expansion in Peyer's patches of FoxP3 + T-reg and follicular T-helper (Thf) lymphocytes ⟶ promote the differentiation of B lymphocytes in IgA producers.

2. CD4+ T lymphocytes. Some metabolites produced by intestinal bacteria, including SCFAs, may promote the differentiation of CD4+ naïve T cells into T-reg. The Th17 subgroup of CD4+ lymphocytes is known for its dual role in both protection against pathogens and inflammatory disorders. The inflammatory propensity of Th17 is largely determined by the type of intestinal microbiota that induces its differentiation.
3. CD8+ (cytotoxic) T lymphocytes, whose effector functions are fundamental in the elimination of intracellular pathogens and tumor cells.
4. Thf lymphocytes are specialized to assist B cells and are crucial for germinal center formation, affinity maturation, and the generation of high-affinity antibody responses.

Cell. Mol. Life. Sci (2020) 77:1497–1509
Front. Microbiol. 6:1085. doi: 10.3389/fmicb.2015.01085

Adaptive immune

1. Microbes in the intestine are sampled by DCs either directly from the lumen or translocation through M cells to the GALT.
2. A combination of signals from the microbes results in phenotypic changes in the DCs and migration to the draining lymph node.
3. DCs promote the activation of various T cell subsets within the MLN and the production of various regulatory cytokines such as IL-10, TGF-β, INFγ, and IL-6.



Cell. Mol. Life. Sci (2020) 77:1497–1509
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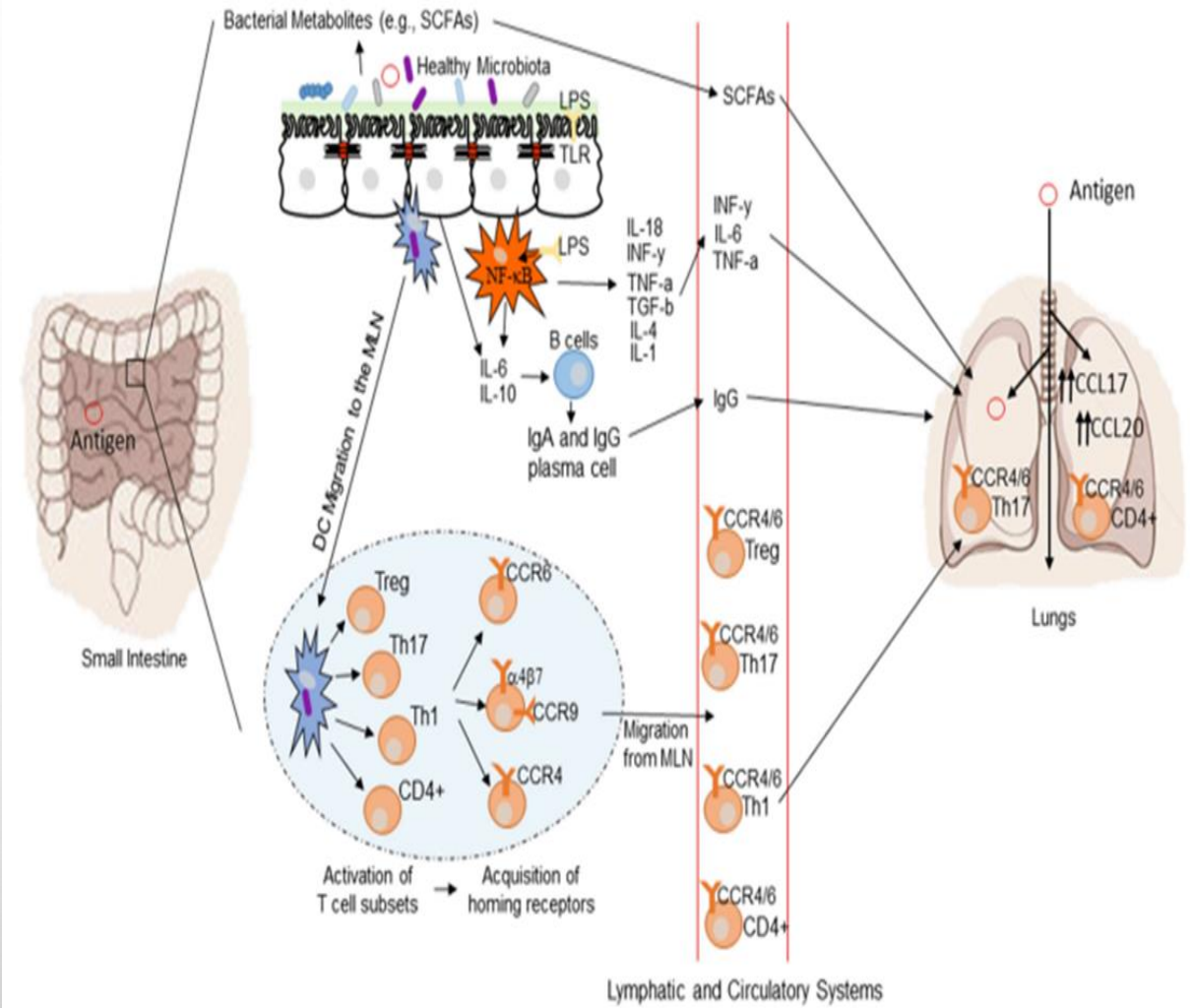
Adaptive immune

4. T cell subsets then acquire immune homing molecules (i.e.CCR6, CCR4, and CCR9).
5. Following immune challenge in the airway, cells activated in the GALT and MLN traffic to the respiratory mucosa via CCR4 or CCR6 where they promote protective and anti-inflammatory responses.
6. Bacterial products such as LPS can bind to TLR present on both intestinal epithelial cells and macrophages, leading to the production of various cytokines and chemokines.



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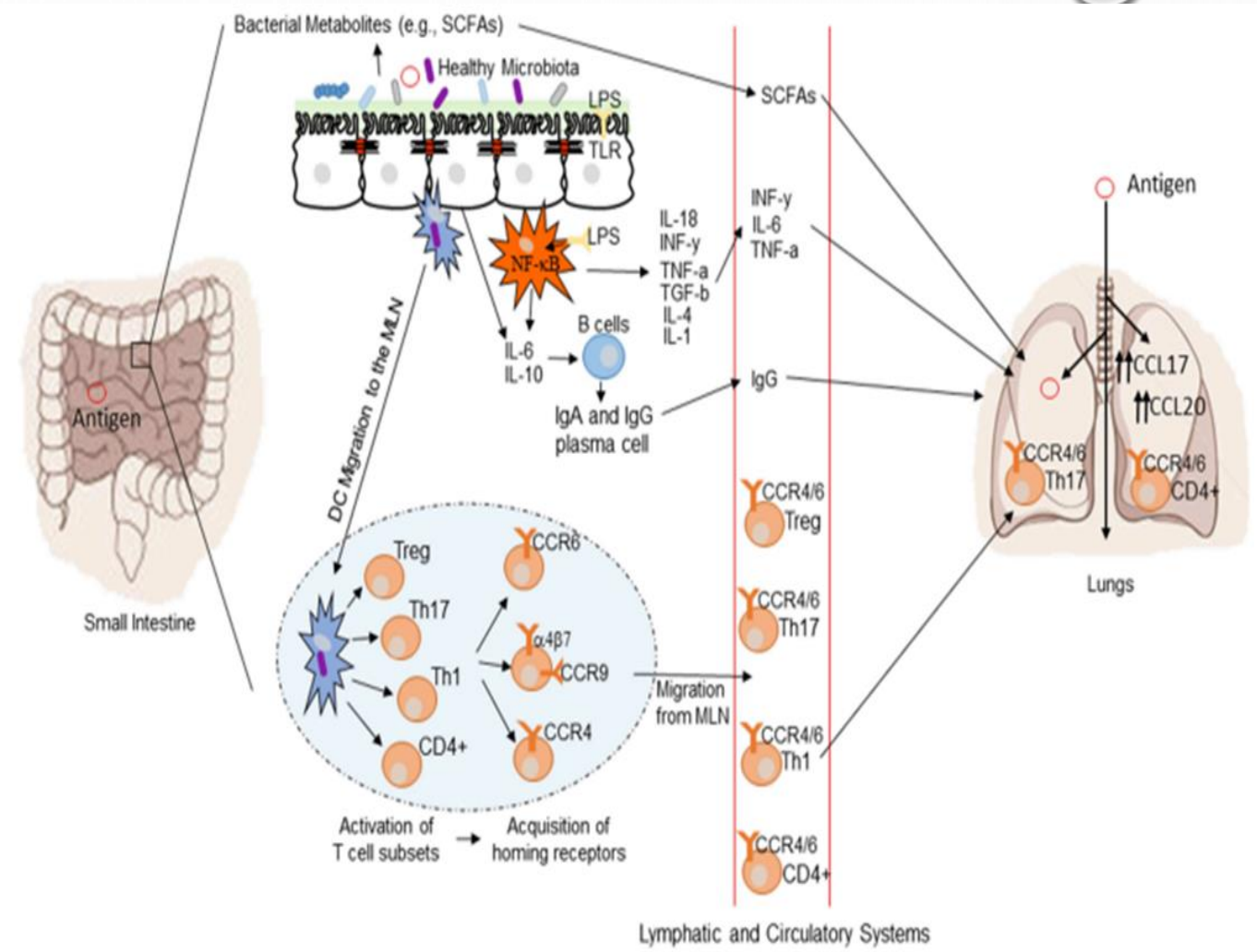
Adaptive immune

7. TLR activation also includes the expression of NF-kB in macrophages.
8. Production of various bacterial metabolites (e.g., SCFAs) also affect the gut-lung axis, as these products are transported to the lung, where they can alter the levels of inflammation.



Cell. Mol. Life. Sci (2020) 77:1497–1509

1. Macrophages, neutrophils, and T and B cells, migrate to infection sites.

2. Form granulomas around *M. tuberculosis*, and restrict its replication (latent TB infection).

3. *M. tuberculosis* can still survive and replicate in granulomas by inhibiting the maturation of phagolysosomes and destructing the patterns of cell death and immune response.

4. When granulomas are impaired due to factors, such as HIV infection, smoking, aging, and malnutrition, *M. tuberculosis* can escape from granulomas and spread to other tissues (active TB infection).

5. Fecal transplantation was shown to reconstitute the gut microbiota, restore anti-TB immunity, and prevent dissemination of TB to other organs.

MiRNAs

1. Small non-coding RNAs
2. Act as post-transcriptional regulators of mRNA by targeting specific RNAs for destruction or by repressing their translation.
3. The regulation of cytokine production and function by miRNA.
4. MicroRNA-21 has been identified as one of the miRNAs in various tumors that may impact the disease, including roles in both innate and adaptive immunity.

- MiR-21 can modulate the responses of macrophages to bacterially derived Toll-like receptor (TLR) agonists including LPS.
- MiR-21 was also shown to be involved in inflammatory responses and regulate the immune responses by targeting programmed cell death 4 (PDCD4).
- MiR-21 can be induced in the lung of multiple asthma models and regulates lung eosinophilia, theTh1/Th2 balance and the prognosis for asthma

Front. Microbiol. 2020.512581

- ✓ MiRNAs show different expression patterns when the gut microbiota is alter.
- ✓ MiR-21 whose expression is remotely controlled by the microbiome directly targets IFN-γ and might in turn inhibit IFN-γ production leading to impaired anti-TB immunity.
- ✓ MiRNAs recently reported to be governed by commensal bacteria, affected NF-kB pathway activity via targeting the IL-23p19 gene.

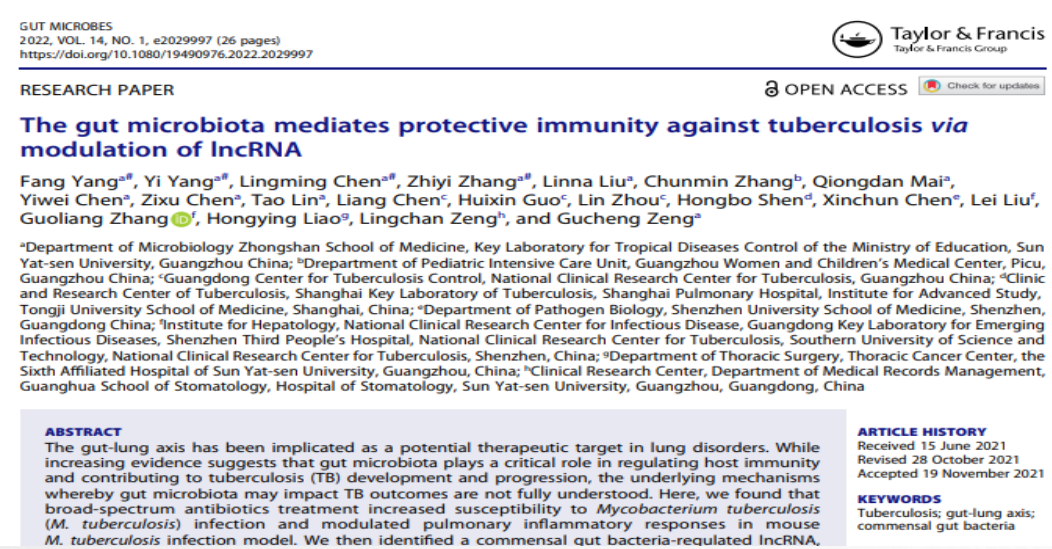
Microbiota regulates host gene expression through modulation of the host miRNA signature.

Gut Microbes 2022, VOL. 14, NO. 1, e2029997

Commensal gut bacteria-regulated- long non-coding RNA (lncRNA-CGB)

- Down-regulated by dysbiosis of gut microbiota during TB infection.
- LncRNA-CGB played a beneficial role in developing immune resistance to TB infection and pathology.
- *Bacteroides fragilis* was a direct regulator of lncRNA-CGB, and oral administration of *B. fragilis* enhanced expression of lncRNA-CGB and promoted anti-TB immunity.
- Genomic knock-out of lncRNA-CGB led to reduced IFN-γ expression and impaired anti-TB immunity.
- LncRNA-CGB interacted with Enhancer of zeste homolog 2 (EZH2) and negatively regulated H3K27 tri-methylation (H3K27Me3) epigenetic programming, leading to enhanced IFN-γ expression.

❖ EZH2 inhibits genes responsible for suppressing tumor development, and blocking EZH2 activity may slow tumor growth



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RESEARCH PAPER

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The gut microbiota mediates protective immunity against tuberculosis *via* modulation of lncRNA

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ABSTRACT

The gut-lung axis has been implicated as a potential therapeutic target in lung disorders. While increasing evidence suggests that gut microbiota plays a critical role in regulating host immunity and contributing to tuberculosis (TB) development and progression, the underlying mechanisms whereby gut microbiota may impact TB outcomes are not fully understood. Here, we found that broad-spectrum antibiotics treatment increased susceptibility to *Mycobacterium tuberculosis* (*M. tuberculosis*) infection and modulated pulmonary inflammatory responses in mouse *M. tuberculosis* infection model. We then identified a commensal gut bacteria-regulated lncRNA,

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KEYWORDS
Tuberculosis; gut-lung axis; commensal gut bacteria

LncRNAs

1. Regulate the expression of inflammatory mediators (IFN) in respiratory viral infection.

2. LncRNA might have immunomodulatory properties for a more effective TB control.

3. Dysbiosis of gut microbiota induced more severe TB infection and identified a series of commensal bacteria-associated lncRNAs, of which, a lncRNA (termed lncRNA-CGB) was significantly down-regulated in *M. Tuberculosis* -infected with disruption of gut bacteria.

mBio. 2018 Sep 18;9(5)

Affecting drug absorption during tuberculosis treatment

- ✓ Antibiotics are a major cause of microbiota perturbation.
- ✓ Antibiotic exposure early in life is a risk factor for the development of asthma, diabetes, and etc. Taking various antibiotics can develop antibiotic-associated diarrhea.
- ✓ Anti-TB therapy could lead to rapid, dramatic changes in the diversity and composition of the microbiota community.
- ✓ Treatment regimens for drug-sensitive TB (6 to 9 months) and drug-resistant TB (up to 2 years) are protracted.

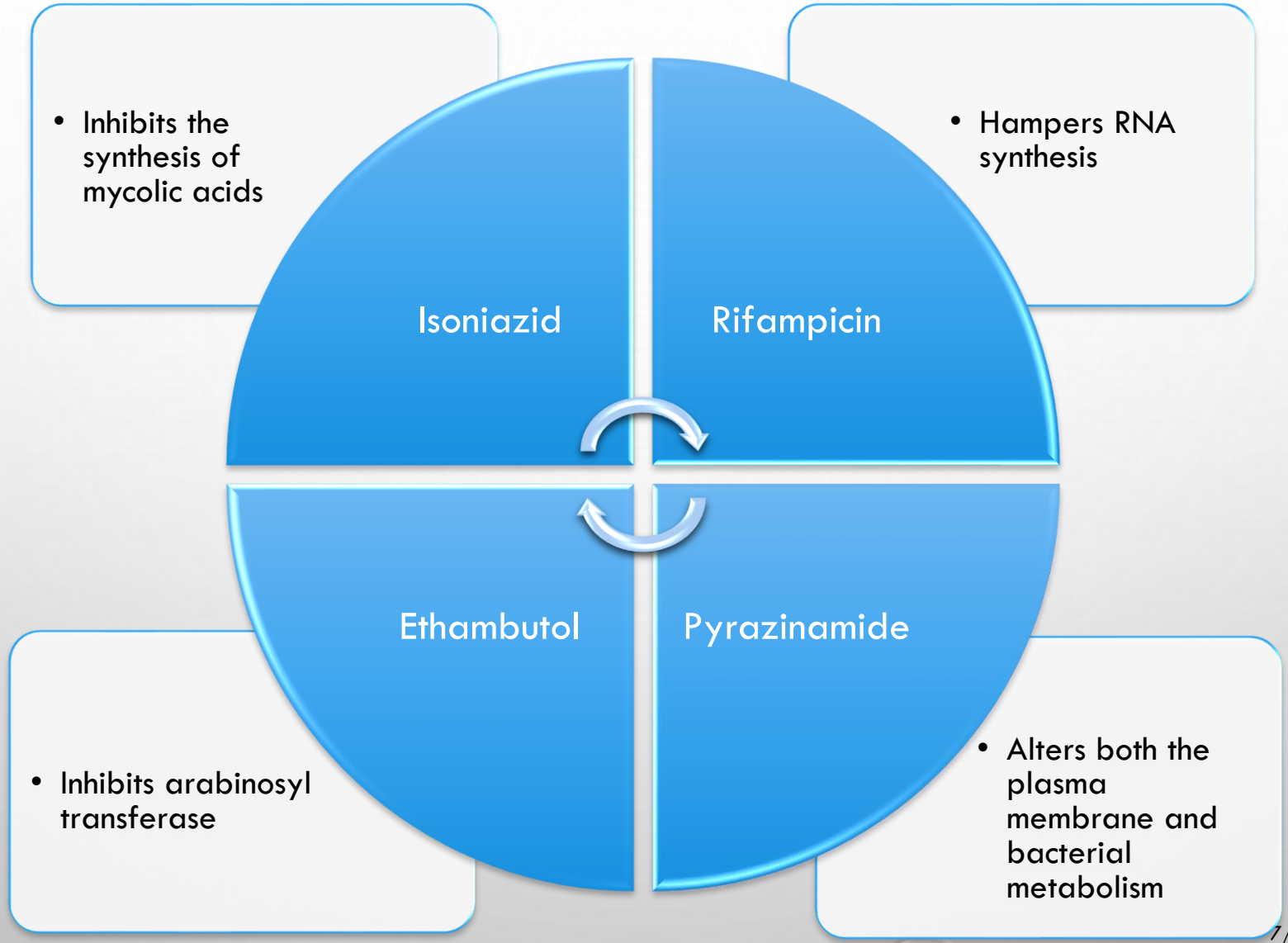
- ✓ Treatment of drug-susceptible TB requires multiple daily administrations of oral antibiotics for a duration of at least 6 months according to World Health Organization guidelines.

- ✓ Only rifampin has a broad-spectrum activity against a wide range of Gram-positive and Gram-negative bacteria. Isoniazid, pyrazinamide, and ethambutol specifically target mycobacterial species, with isoniazid and pyrazinamide being prodrugs that need to be activated by mycobacterium-specific enzymes.

Front. Nutr. 8:626254.
doi: 10.3389/fnut.2021

- ✓ During anti-TB treatment, the abundance of genus *Clostridium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus and Peptococcus* significantly decrease, whereas several members of the *Bacteroides* genus, such as *Bacteroides fragilis and Bacteroides* OTU230 increase. These microbiota alterations could even persist for at least 1.2 years.

- ✓ After 1 week of TB treatment, OTU8 and OTU2972 assigned to the family Erysipelotrichaceae strikingly increase, whereas the rest of the Erysipelotrichaceae family decline.


• Inhibits the synthesis of mycolic acids
Isoniazid
• Hampers RNA synthesis
Rifampicin
• Inhibits arabinosyl transferase
Ethambutol
• Alters both the plasma membrane and bacterial metabolism
Pyrazinamide

Front. Microbiol. 2020.512581

mBio. 2018 Sep 18;9(5)

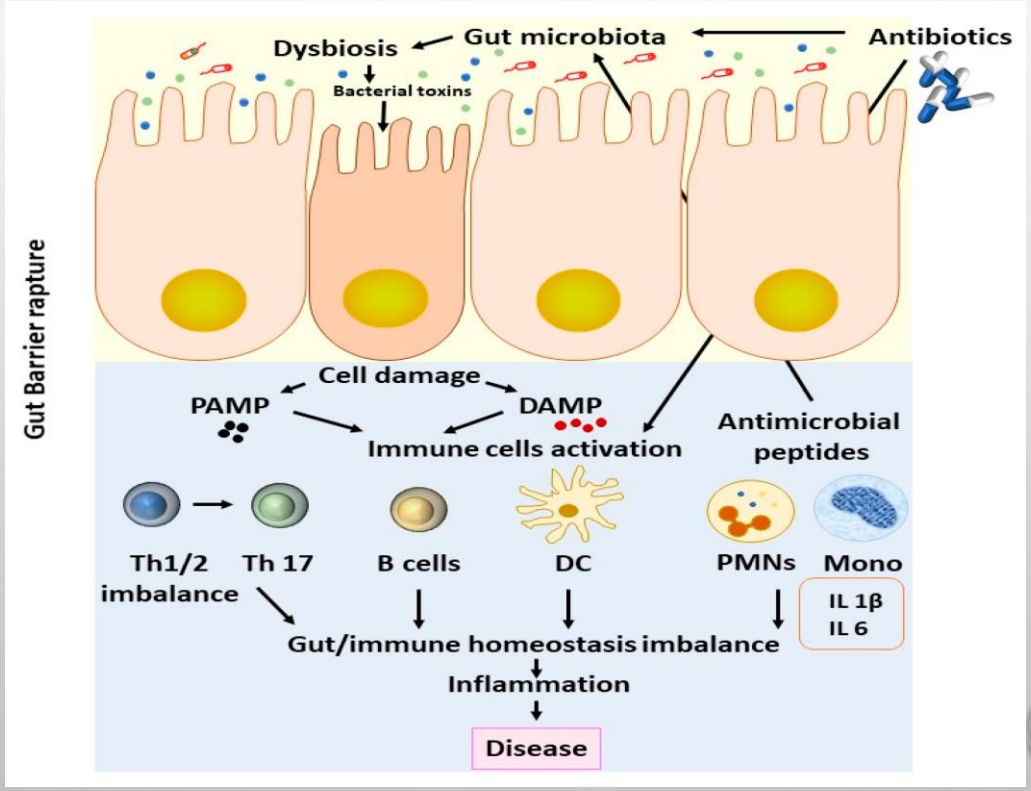
- Studies have shown that conventional TB antibiotic therapy causes a defined and persistent dysbiosis in the intestinal microbiota.

- Treatment with broad-spectrum antibiotics decreased TNFα and IFN-γ production by CD8 + T cells.

- HRZ(E) treatment, both mice and humans have shown strikingly similar effects on the order Clostridiales of the phylum Firmicutes. Clostridia are important players of gut homeostasis and metabolism, particularly via their production of SCFAs.

- Antibiotic-induced dysbiosis inhibited TLR7 signaling, which reduced the secretion of the pro-infammatory cytokines IFN-γ and IL-17, with increase in the levels of IL-4 and IL-10.

- HRZE treatment result in depletion at day 14 of inflammatory response, IFNα response, IFNγ response, TNFα signaling via NFκB, and IL6 JAK STAT3.



mBio. 2018 Sep 18;9(5)

Cellular and Molecular Life Sciences (2020) 77:1497–1509
NATURE COMMUNICATIONS | (2021) 12:1141

- Individuals on anti-TB drug therapy had an enrichment of *Erysipela*, *Clostridium*, *Fusobacterium and Prevotella*, whereas, depletion of *Lactobacillus*, *Coprococcus*, *Ruminococcus and Bifdobacterium* was observed in comparison to the latent TB group.

- The genus *Bacteroides* increased in abundance during anti-TB antibiotics treatment, including *Bacteroides fragilis*, whereas, the population of members within the Clostridiales order, declined.

- In humans, *Bacteroides*, *Faecalibacterium*, *Eubacterium*, *and Ruminococcus* were all biomarkers of dysbiosis when measured more than 1 year posttreatment.

- After an individual is cured by TB antibiotics, their risk for reinfection is increased- up to 4-fold- → Suggesting a possible link between the posttreatment effects of chemotherapy on the microbiome and TB recurrence.

PLoS Pathogens (2021) 17(4): e1009377

After more than 1 year of stopping treatment, the intestinal microbiome of the individuals cured of TB, was clearly distinguishable from the latent TB cohorts, indicating that treatment for TB has a long-lasting efect on microbiome composition

Effects of anti-TB treatment on the host microbiome composition

Location	Specimen	Host	Treatment	Change in microbiota composition	Effects on the immune system	Sequencing technology and data analyisis
Gut	Feces	LTBI ($n = 10$), TB ($n = 28$) TB patients with 1-week anti-TB therapy (TB1, $n = 13$), TB patients with 2-week anti-TB therapy (T2, $n = 10$, cured TB patients (TBc, $n = 10$); healthy individuals ($n = 13$)	INH, RIF, EMB, and PZA	Decrease of *Ruminococcus* and *Faecalibacterium*. Increased abundance of *Bacteroides* species and *Parabacteroides distasonis* in all the treatment groups.	n.d.	16S rRNA gene amplicon (Illumina) sequencing; Ribosomal Database Project (RDP) [Δ]; Mothur v.1.36.1*
	Feces	LTBI ($n = 25$), TB treatment ($n = 19$), cured TB patients ($n = 19$); individuals without *Mtb* infection (IGRA-) as controls ($n = 50$)	INH, RIF, EMB, and PZA	Enrichment of *Erysipelatoclostridium*, *Fusobacterium*, and *Prevotella*; decrease of *Blautia*, *Lactobacillus*, *Coprococcus*, *Ruminococcus*, and *Bifidobacterium* in the TB treatment group. Depletion of *Bacteroides* and overabundance of *Faecalibacterium*, *Eubacterium*, and *Ruminococcus* in cured TB group: *Enterobacter cloacae*, *Phascolarctobacterium succinatutens*, *Methanobrevibacter smithii*, *Bilophila*, and *Parabacteroides* are biomarkers of cured TB patients.	n.d.	16S rRNA gene amplicon (Illumina) sequencing; NCBI refseq_rna database with custom scripts[Δ]; QIIME*/ Shotgun metagenomic Illumina sequencing; Metaphlan2 (microbial species abundances) and HUMAnN2 (functional pathways)
	Feces	MDR-TB treatment group ($n = 6$) and untreated controls ($n = 26$); MDR-TB recovered group ($n = 18$) and untreated control ($n = 28$)	MDR-TB treatment	Bacteroidetes, Cyanobacteria, and Patescibacteria are biomarkers for the recovered group: decrease of Actinobacteria and Firmicutes; increase of Bacteroidetes in recovered group.	n.d.	16S rRNA gene amplicon (Illumina) sequencing; RDP classifier (v 2.2)[Δ]; Mothur*

Summary of microbiome studies performed on animal models of TB and TB patients, investigating the impact of anti-TB treatment on the host microbiome

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MINIREVIEW
Host-Microbe Biology

The Microbiome and Tuberculosis: Early Evidence for Cross Talk

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Summary of antituberculosis treatment-induced alterations in the microbiota

Antibiotic(s)[a]	Effect on intestinal microbiota
HRZ (mice)	Decreases in *Acetivibrio*, *Robinsoniella*, *Alkaliphilus*, *Stomatobaculum*, *Butyricicoccus*, *Acetanaerobacterium*, *Tyzzerella*, *Ruminococcus*, and *Peptococcus* and increase in *Erysipelatoclostridium*
Post-HRZ (mice)	Decrease in *Lactobacillus* and increase in *Barnesiella*, *Porphyromonas*, *Paraprevotella*, *Parasutterella*, and *Desulfovibrio* and *Actinobacteria* genera
HRZE (humans)	Decrease in *Lactobacillus*, *Coprococcus*, *Ruminococcus*, and *Bifidobacterium* and increase in *Erysipelatoclostridium*, *Fusobacterium*, and *Prevotella*
HRZE (humans)	Decrease in *Prevotella* and *Lachnospira*
Post-HRZE (humans)	Decrease in *Bacteroides* and increase in *Faecalibacterium*, *Eubacterium*, and *Ruminococcus*
H alone	Alterations in *Barnesiella* and certain *Clostridium* species
R alone	Decrease in diversity and a number of *Clostridium* species
Z alone	Alterations in *Anaeroplasma* and certain *Clostridium* species

[a]Abbreviations: H, isoniazid; R, rifampin; Z, pyrazinamide; E, ethambutol.

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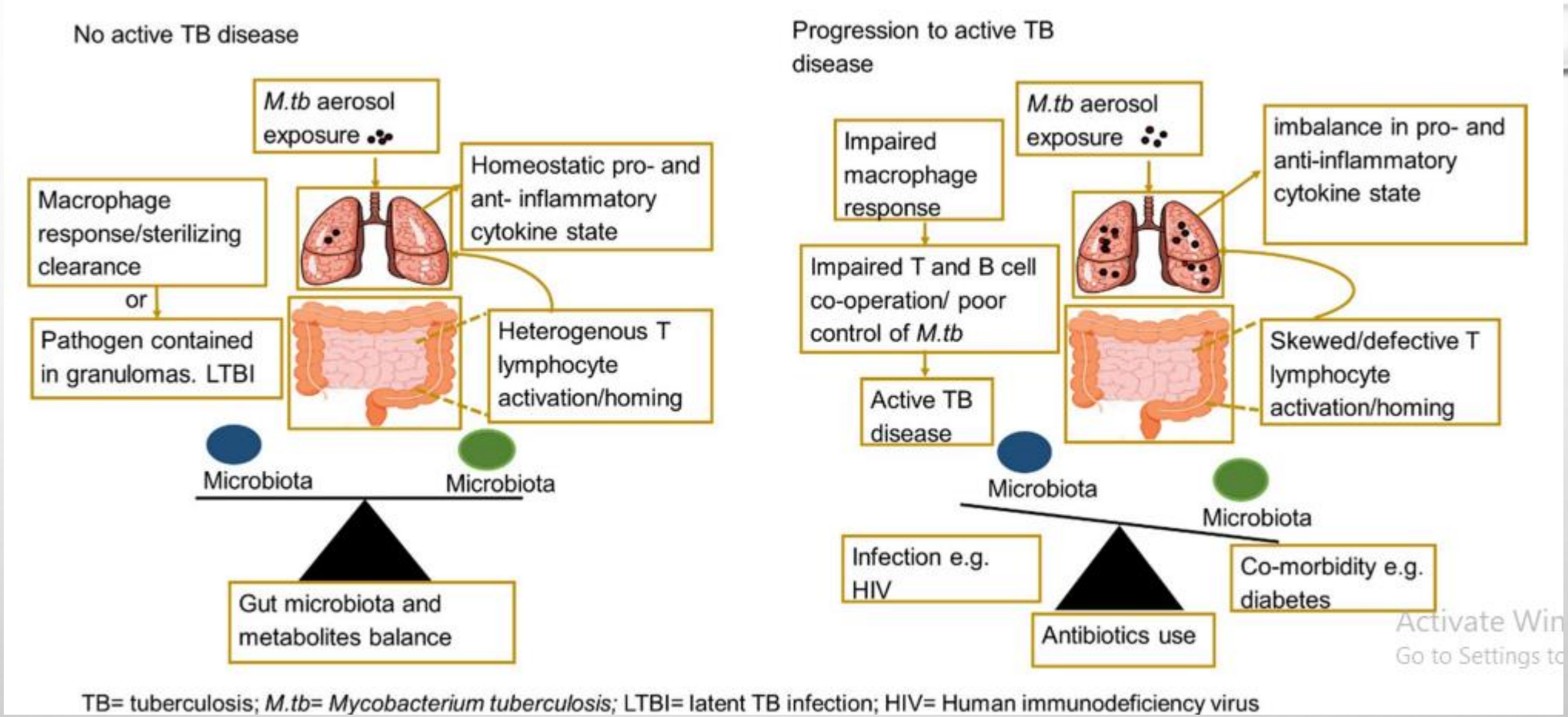


Fig.5 Model for gut microbiome and metabolite regulation of cytokine responses during tuberculosis disease

Front. Immunol. 10:1142.

Gut microbiota regulates mincle mediated activation of lung DCs to protect against *Mtb*

- Gut microbial components serve as ligand for various PRRs present on immune cells and regulates host immunity.

- Mincle (macrophage inducible C-type lectin) also known as Clec4e (C-type lectin domain family 4 member E) or Clecsf9 (C-type lectin superfamily member 9) is a PRR, involved in glycolipids recognition derived from mycobacteria.
- Expressed on DCs, macrophages, B cells and neutrophils

Highly specialized innate cells involved in immune response to *Mtb* infection

✓ Gut commensal *Lactobacillus plantarum* derived cyclopropane-fatty acid α-glucosyl diglyceride (glycolipid)

has been reported to bind and activate mincle.

Front. Immunol. 10:1142.

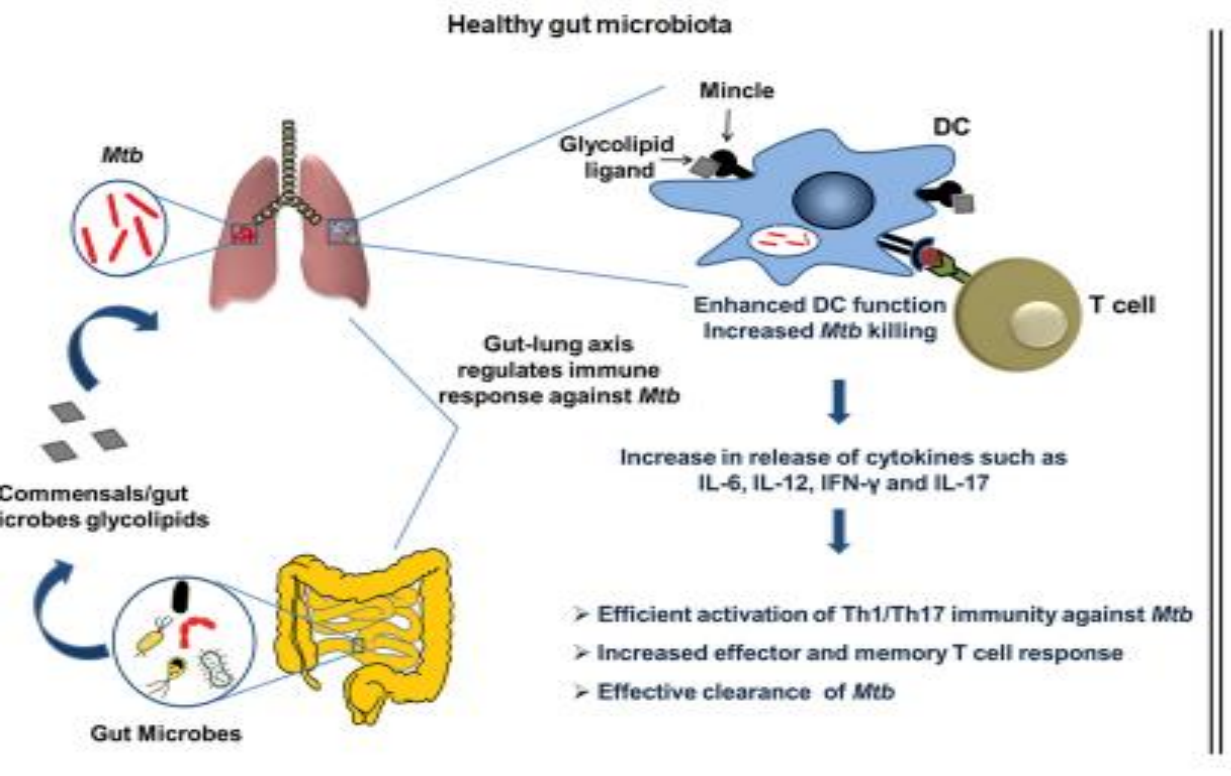
✓ Trehalose-dibehenate (TDB) is a synthetic glycolipid analog known to signal through mincle.

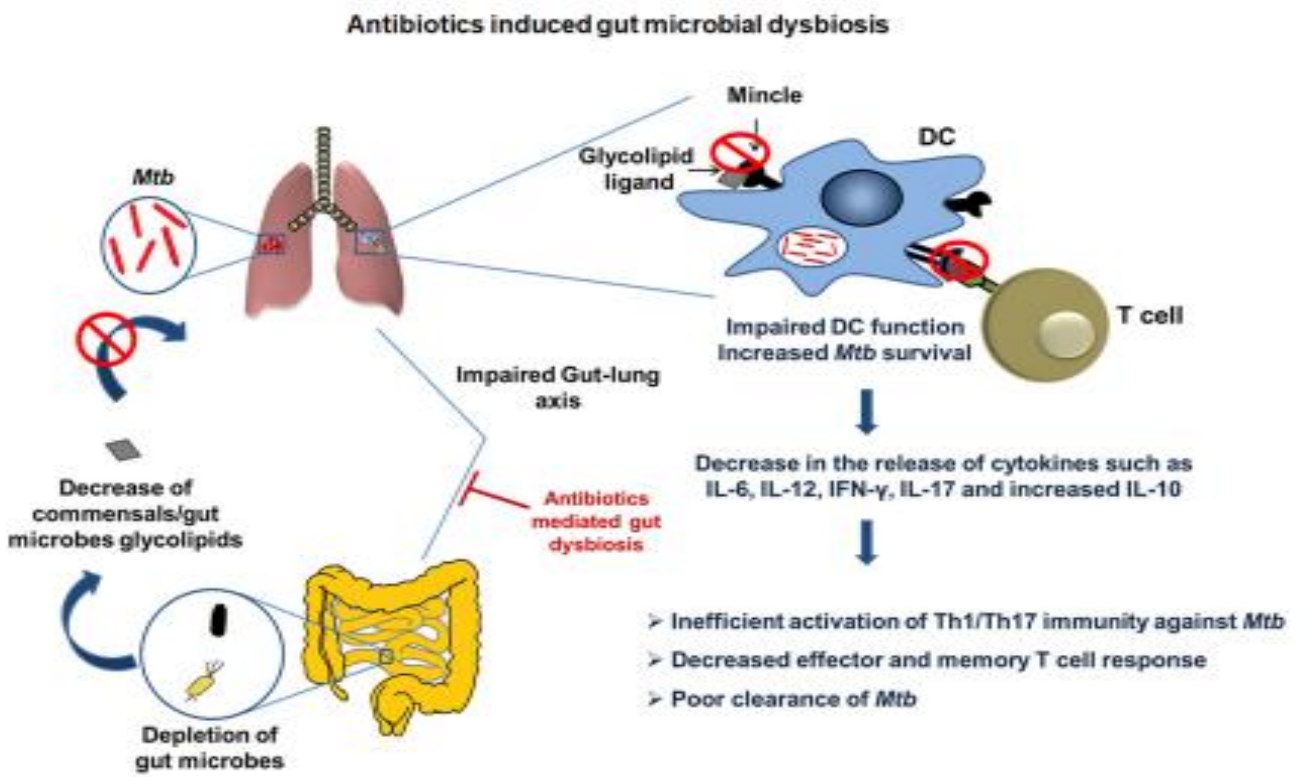
➢ Dysbiosis of gut microbiota abrogated the mincle expression ⟹ compromising DCs function and subsequent T cell response against *Mtb*. These immune defects after Abx treatment could be restored by supplementation with TDB and *Lactobacillus*.

- These DCs with impaired phenotype and functions had reduced ability to activate naïve CD4 T cells, and thus unable to restrict *Mtb* survival.

- In vivo administration of trehalose-6,6-dibehenate (TDB: mincle ligand) efficiently rescued this immune defect by enhancing lung DCs function and subsequent T cell response.

- Supplementation with *Lactobacillus* restored mincle expression on DCs along with *anti-Mtb* response.





During homeostasis, a healthy gut through microbial products such as glycolipids (synthetic analog, TDB that binds mincle) regulates lung immune response during *Mtb* infection. Gut commensals bacteria derived glycolipids reach lung via blood stream. In *Mtb* infected lungs, they bind to the mincle receptor expressed on DCs leading to their activated phenotype and functions such as production of immunoregulatory cytokines (IL-6, IL-12), which in turn elicits the CD4 T cells differentiation to Th1 and Th17 cells via the release of IFN-γ and IL-17 cytokines, respectively. Further, there is generation of memory response and protective immunity against *Mtb* in lungs. This immunoregulation through gut microbiota is disturbed upon the Abx induced dysbiosis. Abx depletes the beneficial commensals population, which is responsible for the impairment of DCs function and hence the dysregulated lung *anti-Mtb* immunity.

Conclusion

- Alterations in the gut microbiome contribute to bias in inter-individual levels of susceptibility to *M.tb* infection or response to TB drug treatment are still emerging. gut microbiome composition could modulate T1/T17 cell–mediated immune responses, which are potentially relevant to TB susceptibility.

- Gut microbiome dysbiosis induced by the protracted anti-TB antibiotics treatment is linked to increased susceptibility to *Mtb* re-infection or TB recrudescence after successful cure.

- Probiotics and postbiotics have exhibited anti-tuberculosis activity *in vitro* and *in vivo*, indicating their potential for application in anti-TB treatment to overcome complications caused by the current use of multiple antibiotics.

- Fecal microbiota transfer (FMT) might serve to augment the treatment and immune control of *M. tuberculosis* infection.

Future perspectives

Detailed analyses of

The role of microbiota in active TB disease, latency, reactivation from latency and clearance with or without antibiotic treatment remains to be thoroughly investigated.

Instead of examining microbiota in TB disease on its own, a more in-depth understanding of their interactions with airway epithelium and the innate and adaptive immune systems will be required.

1. Eribo OA, du Plessis N, Ozturk M, Guler R, Walzl G, Chegou NN. The gut microbiome in tuberculosis susceptibility and treatment response: guilty or not guilty?. Cellular and Molecular Life Sciences. 2020 Apr;77:1497-509.

2. Shahzad M, Andrews SC, Ul-Haq Z. Protocol: Exploring the role of Microbiome in Susceptibility, Treatment Response and Outcome among Tuberculosis Patients from Pakistan: study protocol for a prospective cohort study (Micro-STOP). BMJ Open. 2022;12(6).

3. Hu Y, Feng Y, Wu J, Liu F, Zhang Z, Hao Y, Liang S, Li B, Li J, Lv N, Xu Y, Zhu B and Sun Z (2019) The Gut Microbiome Signatures Discriminate Healthy From Pulmonary Tuberculosis Patients. Front. Cell. Infect. Microbiol. 9:90. doi: 10.3389/fcimb.2019.00090.

4. Negi S, Pahari S, Bashir H and Agrewala JN (2019) Gut Microbiota Regulates Mincle Mediated Activation of Lung Dendritic Cells to Protect Against Mycobacterium tuberculosis. Front. Immunol. 10:1142. doi: 10.3389/fimmu.2019.01142.

5. Gupta N, Kumar R and Agrawal B (2018) New Players in Immunity to Tuberculosis: The Host Microbiome, Lung Epithelium, and Innate Immune Cells. Front. Immunol. 9:709. doi: 10.3389/fimmu.2018.00709.

6. Dumas A, Corral D, Colom A, Levillain F, Peixoto A, Hudrisier D, Poquet Y and Neyrolles O (2018) The Host Microbiota Contributes to Early Protection Against Lung Colonization by Mycobacterium tuberculosis. Front. Immunol. 9:2656. doi: 10.3389/fimmu.2018.02656.

7. Liu Y, Wang J and Wu C (2021). Microbiota and Tuberculosis: A Potential Role of Probiotics, and Postbiotics. Front. Nutr. 8:626254. doi: 10.3389/fnut.2021.626254

8. Comberiati, P.; Di Cicco, M.; Paravati, F.; Pelosi, U.; Di Gangi, A.; Arasi, S.; Barni, S.; Caimmi, D.; Mastrorilli, C.; Licari, A.; et al. The Role of Gut and Lung Microbiota in Susceptibility to Tuberculosis. Int. J. Environ. Res. Public Health 2021, 18, 12220. https://doi.org/10.3390/ ijerph182212220.

9. Namasivayam S, Sher A, Glickman MS, Wipperman MF. 2018. The microbiome and tuberculosis: early evidence for cross talk. mBio 9:e01420-18. https://doi.org/10.1128/mBio .01420-18.

10. Mori G, Morrison M, Blumenthal A (2021) Microbiome-immune interactions in tuberculosis. PLoS Pathog 17(4): e1009377. https://doi.org/ 10.1371/journal.ppat.1009377.

11. Eribo OA, du Plessis N, Ozturk M, Guler R, Walzl G, Chegou NN. The gut microbiome in tuberculosis susceptibility and treatment response: guilty or not guilty?. Cellular and Molecular Life Sciences. 2020 Apr;77:1497-509.

12. Samuelson DR, Welsh DA and Shellito JE (2015) Regulation of lung immunity and host defense by the intestinal microbiota. Front. Microbiol. 6:1085. doi: 10.3389/fmicb.2015.01085.

13. Ruan W, Engevik MA, Spinler JK, Versalovic J. Healthy human gastrointestinal microbiome: composition and function after a decade of exploration. Dig. Dis. Sci. 2020 Mar;65:695-705.

14. Dekaboruah E, Suryavanshi MV, Chettri D, Verma AK. Human microbiome: an academic update on human body site specific surveillance and its possible role. Archives of microbiology. 2020 Oct;202:2147-67.

15. Wang B, Yao M, Lv L, Ling Z, Li L. The human microbiota in health and disease. Engineering. 2017 Feb 1;3(1):71-82.

16. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. Bmj. 2018 Jun 13;361.

17. Yang F, Yang Y, Chen L, Zhang Z, Liu L, Zhang C, Mai Q, Chen Y, Chen Z, Lin T, Chen L. The gut microbiota mediates protective immunity against tuberculosis via modulation of lncRNA. Gut Microbes. 2022 Dec 31;14(1):2029997.

18. Mori G, Morrison M, Blumenthal A (2021) Microbiome-immune interactions in tuberculosis. PLoS Pathog 17(4): e1009377. https://doi.org/ 10.1371/journal.ppat.1009377.