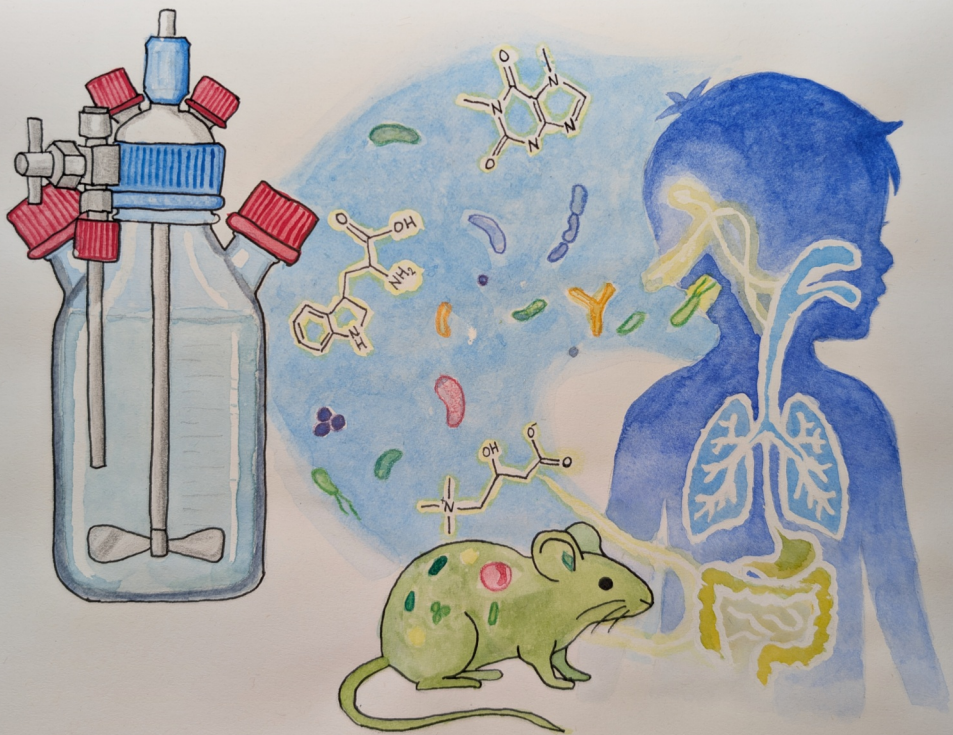


The Gut Microbiota – Host Interaction

from the Clinic
through Mouse Models
into Bioreactors

Ymke de Jong



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Ymke de Jong

Academic dissertation for the Degree of Doctor of Philosophy in Molecular Bioscience at Stockholm University to be publicly defended on Tuesday 26 May 2026 at 09.00 in Vivi Täckholmsalen (Q-salen), Svante Arrheniusväg 20 and online, public link is available at the department website.

Abstract

The interplay between the microbiota and host in health and disease is being extensively studied and the field is expanding exponentially. The microbiota is in symbiosis with its host and, especially during early life, actively influences the development of the host immune system. Disturbances of the gut microbiota during this window of opportunity can alter the microbial metabolism to an extent that it affects the host development for years thereafter. Dysbiosis or shifts in gut microbial composition have been shown to correlate with childhood allergy and asthma development. Together, these observations suggest that the microbiota is one of the driving forces for immune development and long-term memory.

In **Paper I**, we showed that early-life immune and gut microbiota signatures are associated with allergic asthma in young adulthood. By only selecting individuals who were genetically prone to become allergic, we could limit genetic variability and study the association of the early-life microbiota with young adulthood allergic asthma. Microbial composition development during the first 2 years of life differed between individuals who had developed allergic asthma in young adulthood compared to those who did not. Additionally, at age 2, we observed different immunological patterns in both dendritic cells and in peripheral blood mononuclear cells (PBMCs) transcriptomic profiles, with increased RNA processing and reduced immune pathway activity. Interestingly, these differences were less pronounced at 20 years of age, once asthma had developed, pointing to early life as a critical period during which microbiota-immune interactions may influence later asthma risk.

In **Paper II**, we connected specific gut microbial compositions with murine metabolic profiles linking the microbes with the host phenotype. Allergy-associated microbiota (AAM) fecal water stimulation induced inflammatory immune responses in PBMCs, while non-allergy associated microbiota (non-AAM) fecal water stimulation induced regulatory pathways. After transplanting the human early-life allergy associated and non-allergy associated fecal matter into germ-free mice, different metabolic patterns were observed in the intestine and distant organs. Increased levels of long acylcarnitines and sphingolipids were associated with the AAM transplanted mice, while non-AAM mice had higher levels of tryptophan and its derivatives. Our results suggest that the metabolites produced by non-AAM have a regulating, anti-inflammatory effect both *in vitro* and *in vivo*, despite adaptations in the microbial composition due to change of host environment.

In **Paper III**, we focused on isolating the microbiota from any host factors by developing a cost-efficient anaerobic bioreactor for culturing of complex microbial communities derived from intestinal samples. We managed to preserve the majority of the original microbial diversity and were able to establish a stable community to test the influence of antibiotic treatment on the dynamics. The magnitude of the perturbation by the antibiotic treatment depended on the original composition.

In summary, this work provides a glimpse into different ways of studying the gut microbiota-host associations and dynamics in relation to allergy and asthma.

Keywords: *gut microbiota, allergic asthma, anaerobic bioreactor, microbial metabolism, metabolomics, immune development.*

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”Livet är en balansgång.
Ibland faller vi, men det
viktiga är att resa oss
upp igen och fortsätta
gå.”

- Selma Lagerlöf

Populärvetenskaplig sammanfattning

Vi är inte ensamma och kommer aldrig att vara det; vi kommer alltid att vara i sällskap av mer än 100 biljoner bakterier. De flesta av dessa bakterier ingår i vår tarmflora, som hjälper till att bryta ner vår mat och därigenom producera metaboliter. Utöver att fungera som en energikälla bidrar dessa bakteriella metaboliter och bakterierna själva till vår hälsa genom signaler som tränar, aktiverar och påverkar immunförsvaret.

Immunförsvaret är ett komplext nätverk av celler och molekyler som skyddar oss mot patogener och andra främmande ämnen. Under spädbarnstiden är immunförsvaret som en orkester där instrumenten fortfarande lär sig att spela tillsammans; tidiga mikrobiella och miljömässiga signaler fungerar som dirigenten som lär dem att spela i harmoni snarare än disharmoni. Eventuella störningar under denna process, såsom antibiotika eller kostförändringar, kan få långsiktiga effekter. Till exempel har allergier och astma hos barn, vilket är överdrivna immunreaktioner på i övrigt ofarliga ämnen, kopplats till förändringar i tarmfloran tidigt i livet. De exakta mekanismerna, och huruvida denna effekt kvarstår i vuxen ålder, är dock okända.

I denna avhandling kopplar vi samman mikrobiotan tidigt i livet med astma i ung vuxen ålder och utvärderar nya sätt att studera hur dessa mikrober påverkar sin mänskliga värd.

I **delarbete I** undersökte vi sambandet mellan spädbarns tarmhälsa och allergisk astma hos 20-åringar. Genom att analysera avförings- och blodprover från individer med liknande genetisk risk för allergi, fann vi att de som utvecklat astma vid 20 års ålder redan uppvisade tydliga mikrobiella mönster mellan en veckas och två års ålder. Intressant nog observerade vi också tydliga skillnader i deras immunmarkörer vid två års ålder, trots att de framstod som kliniskt friska vid tillfället. Detta tyder på att förändrade mikrobiella profiler under de första två levnadsåren präglar immunförsvaret på ett sätt som kan leda till astma decennier senare.

För att förstå hur dessa bakterier kommunicerar med kroppen tittade vi i **delarbete II** på de specifika metabola fingeravtryck som mikrobiotan lämnat i avföringsproven. Vi fann att bakterier förknippade med allergiutveckling utlöste inflammatoriska reaktioner i immuncellerna, medan "hälsoassocierade" bakterier stimulerade immunreglerande vägar för att hålla nere inflammationen. Vidare transplanterade vi dessa mänskliga mikrobiella samhällen till möss och såg tydliga metabola mönster, inte bara i tarmen utan även i avlägsna organ. Möss med allergiassocierade mikrober hade lägre nivåer av antiinflammatorisk tryptofan, men högre nivåer av inflammationsrelaterade sfingolipider. Detta bekräftar att de metaboliter som produceras av hälsoassocierade bakterier har en positiv effekt på värden.

Slutligen, i **delarbete III**, ville vi studera dessa mikrober utan påverkan från värden. För att göra detta utvecklade vi en kostnadseffektiv anaerob bioreaktor som efterliknar tarmens syrefria miljö. Vi lyckades odla komplexa mikrobiella samhällen i detta system och bevarade många av de ursprungliga bakterierna från avföringsproven. Vi upptäckte att förändringar i bakteriesamhället, orsakat av antibiotika, i hög grad beror på bakteriesamhällets

ursprungliga sammansättning.

Sammantaget ger detta arbete en inblick i de många sätt vi kan studera hur tarmfloran påverkar vår hälsa. Genom att förstå denna dynamik kommer vi närmare att hitta sätt att förebygga allergi och astma innan de faktiskt utvecklas.

Popular science summary

We are not alone and will never be, we will always be accompanied by more than 100 trillion bacteria. Most of these bacteria are part of our gut microbiota, which helps digest our food and thereby produce metabolites. Besides functioning as an energy source, these bacterial metabolites and the bacteria themselves contribute to our health with signals that train, trigger and influence the immune system.

The immune system is a complex network of cells and molecules that protects us from pathogens and other foreign objects. In infancy, the immune system is an orchestra of instruments still learning to play together; early microbial and environmental cues act as the conductor that teaches them to perform in harmony rather than discord. Any disruptions during this process, such as antibiotics or changes in diet, can have long-term effects. For example, childhood allergies and asthma, which are excessive immune reactions to otherwise harmless substances, have been linked to early-life changes in the gut microbiota. However, the exact mechanisms, and whether this effect persists into adulthood, are unknown.

In this thesis, we connect the early-life microbiota with asthma in young adulthood and evaluate new ways to study how these microbes influence their human host.

In **paper I**, we investigated the link between infant gut health and allergic asthma in 20-year-olds. By analyzing fecal and blood samples from individuals with a similar genetic risk for allergy, we found that those who developed asthma by age 20 already showed distinct microbial patterns between one week and two years of age. Interestingly, we also observed clear differences in their immune markers at age two, even though they appeared clinically healthy at the time. This suggests that altered microbial profiles in the first two years of life prime the immune system in a way that may lead to asthma decades later.

To understand how these bacteria communicate with the body, we looked at the specific metabolic footprints left by the microbiota in the fecal samples in **paper II**. We found that bacteria associated with allergy development triggered inflammatory responses in the lab, while “healthy” associated bacteria stimulate immune regulatory pathways to keep inflammation down.

We further transplanted these human microbial communities into mice and saw distinct metabolic patterns not just in the gut, but also in distant organs. Mice with allergy-associated microbes had lower levels of anti-inflammatory tryptophan, while higher levels of inflammation related sphingolipids. This confirms that the metabolites produced by health-associated bacteria have a positive effect on the host.

Finally, in **paper III**, we wanted to study these microbes without influence from the host. To do this, we developed a cost-efficient anaerobic bioreactor that mimics the gut oxygen-free environment. We successfully grew complex microbial communities in this system, preserving many of the original bacteria retrieved from fecal samples. We discovered that the severity of the disruption caused by antibiotics depends heavily on the original makeup

of the bacterial community.

Taken together, this work provides a glimpse into the many ways we can study how the gut microbiota influences our health. By understanding these dynamics, we move closer to finding ways to prevent allergy and asthma before they actually begin.

Populairwetenschappelijke samenvatting

We zijn niet alleen en zullen dat ook nooit zijn; we zullen altijd worden vergezeld door meer dan 100 biljoen bacteriën. De meeste van deze bacteriën maken deel uit van onze darmmicrobiota, die helpen bij het verteren van ons voedsel en daarbij metabolieten produceren. Naast hun functie als energiebron dragen deze bacteriële metabolieten en de bacteriën zelf bij aan onze gezondheid met signalen die het immuunsysteem trainen, activeren en beïnvloeden.

Het immuunsysteem is een complex netwerk van cellen en moleculen dat ons beschermt tegen pathogenen en andere lichaamsvreemde stoffen. Tijdens de babytijd is het immuunsysteem als een orkest waarvan de instrumenten nog moeten leren samenspelen; vroege microbiële en omgevingsignalen fungeren als de dirigent die ze leert in harmonie te spelen in plaats van in strijd. Elke verstoring in dit proces, zoals antibiotica of veranderingen in het dieet, kan langetermijneffecten hebben. Zo zijn allergieën en astma bij kinderen, wat in feite overmatige immuunreacties op verder onschadelijke stoffen zijn, gekoppeld aan veranderingen in de darmmicrobiota op jonge leeftijd. De exacte mechanismen, en de vraag of dit effect aanhoudt tot in de volwassenheid, zijn echter nog onbekend.

In dit proefschrift leggen we het verband tussen de vroege microbiota en astma op jongvolwassen leeftijd en evalueren we nieuwe manieren om te bestuderen hoe deze microben hun menselijke gastheer beïnvloeden.

In **artikel I** onderzochten we het verband tussen de darmgezondheid van zuigelingen en allergische astma bij 20-jarigen. Door ontlastings- en bloedmonsters te analyseren van individuen met een vergelijkbaar genetisch risico op allergie, ontdekten we dat degenen die op hun 20^e astma hadden ontwikkeld, tussen de leeftijd van één week en twee jaar al duidelijke afwijkende microbiële patronen vertoonden. Interessant genoeg zagen we op tweejarige leeftijd ook duidelijke verschillen in hun immuunmarkers, ook al leken ze op dat moment klinisch gezond. Dit suggereert dat een veranderd microbiële profiel in de eerste twee levensjaren het immuunsysteem zodanig vormt dat het decennia later tot astma kan leiden.

Om te begrijpen hoe deze bacteriën met het lichaam communiceren, hebben we in **artikel II** gekeken naar de specifieke metabole vingerafdrukken die de microbiota achterliet in de ontlasting. We ontdekten dat bacteriën die geassocieerd worden met de ontwikkeling van allergieën ontstekingsreacties uitlokten in het laboratorium, terwijl "gezondheidsgeassocieerde" bacteriën juist immuunregulerende trajecten stimuleren om ontstekingen te onderdrukken.

Verder hebben we deze menselijke microbiële gemeenschappen getransplanteerd naar muizen, waarbij we duidelijke metabole patronen zagen in niet alleen de darmen, maar ook in andere organen. Muizen met allergie-geassocieerde microben hadden lagere concentraties van het ontstekingsremmende tryptofaan, en juist hogere concentraties van ontstekingsgerelateerde sfingolipiden. Dit bevestigt dat de metabolieten geproduceerd door

gezondheidsgeassocieerde bacteriën een positief effect hebben op de gastheer.

Ten slotte wilden we in **artikel III** deze microben bestuderen zonder invloed van de gastheer. Hiervoor hebben we een kostenefficiënte anaerobe bioreactor ontwikkeld die de zuurstofvrije omgeving van de darm nabootst. We zijn erin geslaagd om complexe microbiële gemeenschappen in dit systeem te kweken, waarbij veel van de oorspronkelijke bacteriën uit de ontlastingsmonsters behouden bleven. We ontdekten dat de ernst van de verstoring door antibiotica sterk afhangt van de oorspronkelijke samenstelling van de bacteriële gemeenschap.

Samenvattend biedt dit werk inzicht in de vele manieren waarop we kunnen bestuderen hoe de darmmicrobiota onze gezondheid beïnvloedt. Door deze dynamiek te begrijpen, komen we dichterbij het vinden van strategieën om allergieën en astma te voorkomen voordat ze daadwerkelijk ontstaan.

Scientific summary

The interplay between the microbiota and host in health and disease is being extensively studied and the field is expanding exponentially. The microbiota is in symbiosis with its host and, especially during early life, actively influences the development of the host immune system. Disturbances of the gut microbiota during this window of opportunity can alter the microbial metabolism to an extent that it affects the host development for years thereafter. Dysbiosis or shifts in gut microbial composition have been shown to correlate with childhood allergy and asthma development. Together, these observations suggest that the microbiota is one of the driving forces for immune development and long-term memory.

In **Paper I**, we showed that early-life immune and gut microbiota signatures are associated with allergic asthma in young adulthood. By only selecting individuals who were genetically prone to become allergic, we could limit genetic variability and study the association of the early-life microbiota with young adulthood allergic asthma. Microbial composition development during the first 2 years of life differed between individuals who had developed allergic asthma in young adulthood compared to those who did not. Additionally, at age 2, we observed different immunological patterns in both dendritic cells and in peripheral blood mononuclear cells (PBMCs) transcriptomic profiles, with increased RNA processing and reduced immune pathway activity. Interestingly, these differences were less pronounced at 20 years of age, once asthma had developed, pointing to early life as a critical period during which microbiota-immune interactions may influence later asthma risk.

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In **Paper III**, we focused on isolating the microbiota from any host factors by developing a cost-efficient anaerobic bioreactor for culturing of complex microbial communities derived from intestinal samples. We managed to preserve the majority of the original microbial diversity and were able to establish a stable community to test the influence of antibiotic treatment on the dynamics. The magnitude of the perturbation by the antibiotic treatment depended on the original composition.

In summary, this work provides a glimpse into different ways of studying the gut microbiota-host associations and dynamics in relation to allergy and asthma.

List of scientific papers included in the thesis

- I. **Ymke A. de Jong**[#], Isabella Badolati[#], Ulrika Hellberg[#], Katarína Bankóová, Caroline Nilsson, and Eva Sverremark-Ekström. 2026. "Asthma in Young Adults at High Risk for Allergies Is Traced Back to Immune and Microbiota Signatures in Early Childhood." *Clinical & Experimental Allergy*. 2026;1-4. <https://doi.org/10.1111/cea.70283>
- II. **Ymke A. de Jong**, Rana M. Seren, Vida Ramšak Marčeta, Antonio Checa, Dagbjort H. Petursdóttir, Isabella Badolati, Claudia Moeckel, Omneya Ahmed Osman, Eva Hell, Douglas L. Huseby, Diarmaid Hughes, Craig E. Wheelock, Sarahi L. Garcia, Klas I. Udekwu, Khaleda R. Qazi and Eva Sverremark-Ekström. Impact of early-life human microbiota on the murine host metabolome: insights from a two-generation HMA mouse model and implications for allergic disease. *BMC Microbiology*. 2025;25:575. <https://doi.org/10.1186/s12866-025-04321-9>
- III. **Ymke A. de Jong**, Pilar López Hernández, Paulina Pastuszek, Douglas Huseby, Diarmaid H. Hughes, Eva Sverremark-Ekström, Stefan Bertilsson, Klas I. Udekwu. Establishment of Human Gut Microbiota into Bioreactors shows a clear Niche Adaptation. *Manuscript*.

[#]Authors contributed equally

Additional scientific papers not included in the thesis

- I. Isabella Badolati, **Ymke de Jong**, Carina Uhl, Josefin Ullberg, Marleen Joustra, Ulrika Lorentzon Fagerberg, Caroline Nilsson, Anna Asarnej, Eva Sverremark-Ekström. One Year of Oral Immunotherapy Impacts the Gut Microbiota and Plasma Metabolome of Peanut-Allergic Young Children. *Clinical & Experimental Allergy*. 2025;55:340-343. <https://doi.org/10.1111/cea.14607>
- II. Khaleda Rahman Qazi, Dhanapal Govindaraj, Magalí Martí, **Ymke de Jong**, Georg Bach Jensen, Thomas Abrahamsson, Maria C. Jenmalm, Eva Sverremark-Ekström. Impact of Extreme Prematurity, Chorioamnionitis, and Sepsis on Neonatal Monocyte Characteristics and Functions. *Journal of Innate Immunity*. 2024;16:470-488. <https://doi.org/10.1159/000541468>

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List of abbreviations

AAM	Allergy-Associated Microbiota
AhR	Aryl hydrocarbon Receptor
AMP	Antimicrobial peptide
APC	Antigen-presenting Cell
CFU	Colony forming units
DC	Dendritic Cell
DEGs	Differential Expressed Genes
DH	Double Heredity
FcεRI	High-affinity IgE receptor
FMT	Fecal Microbial Transplant
GC	Goblet Cell
GF	Germ-Free
GI	Gastrointestinal
GPCR	G protein-coupled receptor
HDAC	Histone Deacetylase
HMA	Human Microbiota-Associated
HMOs	Human Milk Oligosaccharides
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate Lymphoid Cell
IML	Inner Mucus Layer
LBP	LPS-Binding Protein
LPS	Lipopolysaccharide
M _φ	Macrophage
MC	Mast Cell
MHC	Major Histocompatibility Complex
NA	No Asthma
NH	No Heredity
NK	Natural Killer
non-AAM	non-allergy-associated microbiota
PBMC	Peripheral Blood Mononuclear Cell
PRR	Pattern Recognition Receptor
rRNA	Ribosomal RNA
SCFA	Short-Chain Fatty Acid
sIgE	Specific immunoglobulin E
SL	Sphingolipid
SNP	Single Nucleotide Polymorphisms

TCR	T-cell Receptor
T _{FH}	T follicular helper (cell)
T _H	T helper (cell)
TLR	Toll-like receptor
T _{REG}	Regulatory T (cell)
TSLP	Thymic Stromal Lymphopietin
Tph	Tryptophan hydroxylases
T1	Type 1 immune pathways
T2	Type 2 immune pathways
T3	Type 3 immune pathways

List of bacteria

Many bacteria have been assigned new names and have been reclassified during the last 10 years. Here is a list of the current classification (bold) and synonyms. The current classification is used in the kappa, while a mixture might be found in the different papers.

Phyla

-ota, ending to denote a phylum (1)

- **Actinomycetota** – Actinobacteria
- **Bacillota** – Firmicutes
- **Bacteroidota** – Bacteroidetes
- **Pseudomonadota** – Proteobacteria

Family

-aceae ending to denote a family

- **Oscillospiraceae** – Ruminococcaceae

1 Introduction

The relationship between bacteria and humans has been ambiguous throughout the years. Initially bacteria were thought to be bad, and the field of microbiology was dominated by the Germ Theory, trying to find ways to eradicate bacteria. When Theodor Escherich discovered a specific bacterium, later defined as *Escherichia Coli* (2), in the gut of both healthy children and in children with diarrhea it opened the thought of bacteria not always being bad and instead having a symbiotic function. This subsequently led to identification of several other members of the gut microbiota and the recognition of bacteria being a natural part of the human body in the early 20th century. Due to the challenge of culturing anaerobic bacteria, the field did not truly flourish until sixty years later. A turning point occurred when the true complexity of the gut microbiota became visible with the help of microscopic images of rat intestines (3). Finally, in 1992, a telling name was given to the intestinal microbiota, "the neglected human organ", strengthening its position in association to health and disease (4). As sequencing technology advanced and became more affordable and accessible, microbiota research rapidly expanded and further accelerated by the launch of the Human Microbiome Project in 2007 (5).

During the beginning of the 21st century, microbiota research has focused on finding out who is living inside us. With time this shifted towards identifying correlations between composition and health and disease and now mechanisms of these interactions are being studied.

The concept of interactions between the microbiota and the host has two sides. We have the microbiota itself, its ecology, the metabolites it produces, how it interacts and develops within. On the other side we have the host physiology, the immune system, endocrine system, metabolic system etc. In my thesis, I have looked at several parts of this context. Starting in the clinic I explored the long-term effects of the early-life microbiota composition on the development of allergic asthma in young adults. With the human microbiota-associated (HMA) mouse model, I studied the influence the microbiota has on its host by using genetically similar mice, thereby limiting the human genetic background. Finally, with the bioreactors I created the possibility to look at the microbiota itself, free from the host influences, but able to test environmental perturbations such as antibiotics.

I wish you a pleasant reading, and I hope you appreciate the world of microbes all around us.

1.1 The microbiota

We are not individuals, but ecosystems.

We are walk-in zoos.

— Adapted from Bill Bryson (6)

From birth, all organisms are colonized with a consortium of bacteria collectively known as the microbiota. These microbial cells inhabit every mucosal surface and the skin, where environmental factors, such as oxygen levels, humidity and nutrient availability, vary by anatomical site. These variations create distinct ecological niches, resulting in unique biogeographical "footprints" of microbial composition (7–9). The gut microbiota consists of at least 500-1000 distinct species, with even greater diversity at strain level (8).

Together with viruses, fungi, and protozoa, these bacteria constitute a collective genome known as the microbiome, contributing to an enormous reservoir of metabolic functions and physiological flexibility for the host. This complex and dynamic ecosystem of microorganisms plays a fundamental role in regulating essential processes within metabolism (10), immunity (11), and neuroendocrinology (12). In this chapter, I focus on the characteristics of the human microbiota.

1.1.1 The intestinal environment

The gastrointestinal (GI) tract hosts the most complex and densely populated microbial ecosystem in the human body. Here, bacteria ferment dietary substrates, influence immune development, protect against pathogens, and produce metabolites essential for host physiology. The composition of the gut microbiota is complex and varies along both the longitudinal and transverse axes of the intestine, reflecting differences in pH, oxygen levels, flow rate, nutrient availability, and mucus structure (**Fig. 1a**) (13,14).

The small intestine is composed of duodenum, jejunum, and ileum. The duodenum is characterized by elevated levels of oxygen, antimicrobial peptides (AMPs), bile acids, and a pH of ~6 (15). The AMPs and relatively fast flow (16) prevent bacteria from colonizing the host epithelium, keeping numbers down to $\sim 10^1$ - 10^3 colony forming units (CFU)/mL. Facultative anaerobes including members of the Pseudomonadota and *Streptococcus* species as well as anaerobic, but acidic tolerant *Prevotella* are the most common bacteria in this first part of the small intestine (14).

As oxygen is consumed by the bacteria and gastric acid gets diluted (pH ~7,5), the environment becomes favorable for additional bacteria in the jejunum and ileum (15). The transit time is still short, and some oxygen is still present, creating a niche for facultative anaerobic bacteria that can adhere to tissue or mucus, such as segmented filamentous bacteria. Despite counting between $\sim 10^4$ - 10^{11} CFU/mL bacteria, the diversity is still low, including *Streptococcaceae*, *Veillonellaceae*, *Enterobacteriaceae* (17), and *Clostridium* species and coliforms (18).

Further down the intestine, the cecum and colon are densely populated with a high variety of bacteria with an estimate of 10^{11} - 10^{12} CFU/mL. Initially, in the proximal cecum, the pH decreases (pH ~6,3) as fiber and complex polysaccharides are being fermented into short-chain fatty acids (SCFAs) by members of the *Bacteroidaceae* and *Clostridiaceae* families (14). Later, the pH rises back to a more neutral 6,5-7. Both the cecum and colon are strictly anaerobic and have a slow flow; compared to a transit time of 2-6 hours in the small intestine, it takes 10-59 hours from the cecum to the rectum. Most parts of the gut epithelium are covered in mucus, with the inter-fold regions containing greater amounts. This layer of mucus creates a barrier between the gut lumen and the host tissue, but it also creates an environment where *Lachnospiraceae* and *Oscillospiraceae* have found their niche (13). In general, a high concentration of Bacteroidota, Pseudomonadota and Bacillota are observed in the colon. These phyla are mainly represented by the following families: *Bacteroidaceae*, *Enterobacteriaceae*, *Lachnospiraceae*, *Enterococcaceae* and *Oscillospiraceae*.

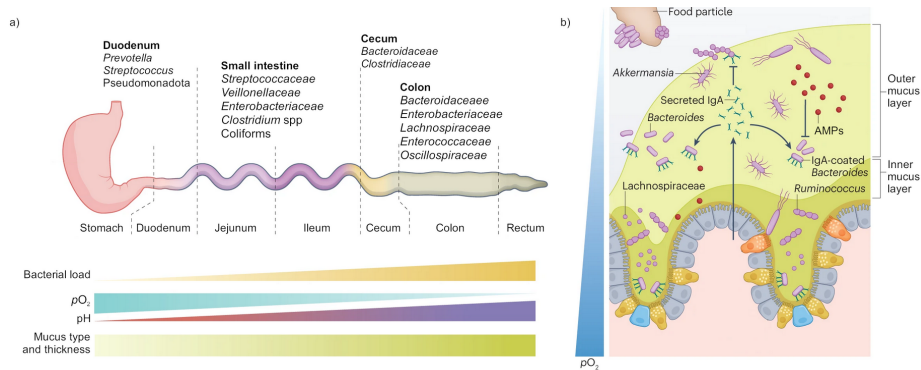


Figure 1: Biogeography of the main gut bacteria in the gastrointestinal tract. a) longitudinal, b) transversal. Adapted from McCallum & Tropini (2024) (14,19).

Even within each longitudinal compartment of the intestine, there are transverse differences between the lumen and closer to the tissue. In the lumen, bacteria are constantly getting pushed forward and flushed out. In the large intestine, the outer mucus layer serves as a niche for certain bacteria to bind. Despite the presence of secreted immunoglobulin A (IgA) and AMPs, *Lachnospiraceae*, *Ruminococcaceae*, *Akkermansia muciniphila* and some *Bacteroides* species have found their niche (13,14). The inner mucus layer (IML) is instead dense, aerobic, and protects against pathogen invasion and only selected bacteria can penetrate (Fig. 1b) (14).

1.1.2 The gut microbiota from neonatal to adult

The composition of the gut microbiome is not stable, but changes over time influenced by delivery mode, diet, disease, lifestyle, and other environmental inputs (Fig. 2) (19–23).

The initial assembly of the human microbiome begins at birth. During this early time

point, the composition of the microbiota is based on the priority effect, where the first colonizing species define the successional trajectory of the ecosystem (21,24,25). This early colonization is shaped mainly by the bacteria dispersed during delivery mode (24,26) and the baby's first contact with the mothers' gut, vaginal and skin microbiota (27). Infants born via cesarean section have an enriched abundance of *Bacillota*, and bacteria originating from skin and oral niches, including *Klebsiella*, *Citrobacter*, *Leclercia*, *Raoultella* (24), *Veillonella*, *Faecalibacterium* (28), *Enterobacter*, *Haemophilus*, *Staphylococcus*, and *Streptococcus* (26). In contrast, the gut microbiota of vaginally delivered infants is characterized by an enrichment of *Bacteroides*, *Bifidobacterium*, *Parabacteroides*, and *Escherichia-Shigella* species (24,26,28). The difference in composition seen between vaginally and cesarean section delivered children is mainly seen in the first weeks-months of life (24,26,28).

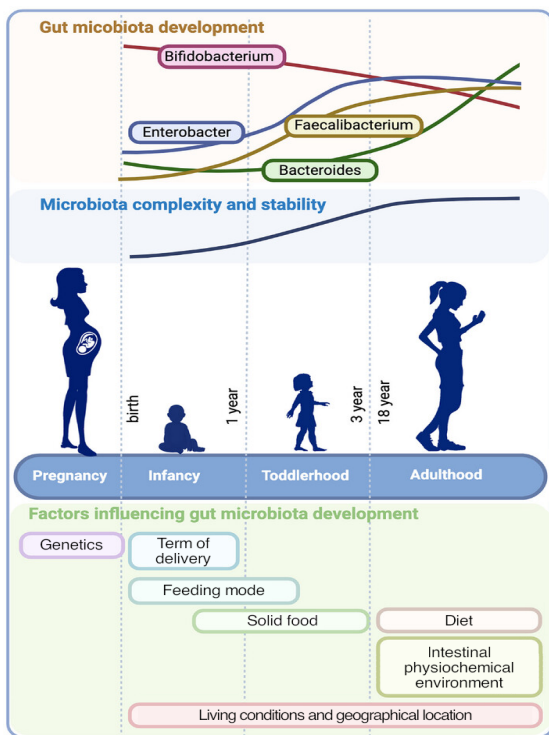


Figure 2. Schematic illustration of the gut microbiota development from delivery to young adulthood. The dynamics of the main bacterial genera are pictured as well as the most important environmental influences. Adapted from Schoultz et al. (2025).

During the first year of life, the infant's microbiota is shaped mainly by the diet, while other environmental factors such as siblings (29), pets (28), and daycare are of lesser influence (30). Breast or formula feeding during the initial months, and later the introduction of solid food, as well as the duration of the breast-feeding in combination with solid food, have a substantial impact on the composition (24,26,31). In breast-fed infants, the presence of human milk oligosaccharides (HMOs) favors a low-diversity community dominated by

Bifidobacterium longum, *Lactobacillus*, *Lacticaseibacillus* and *Bacteroides* (26,32–34). Formula fed infants, on the other hand, have a more diverse composition with *Clostridium difficile*, *Granulicatella adiacens*, *Citrobacter* spp., *Enterobacter cloacae*, *Bilophila wadsworthia* (26) and *Enterobacteriaceae* (19). During weaning, diversity increases more towards an adult-like composition and becomes enriched in *Bacteroides*, *Bilophila*, *Roseburia*, *Clostridium*, *Anaerostipes* (26), and *Lachnospiraceae* family including, *Pseudobutyrvibrio*, *Lachnobacterium*, *Roseburia*, and *Blautia* (24), while species within the *Bifidobacteria*, *Staphylococcus*, *Streptococcus* (24), *Veillonella* and *Bifidobacterium* genera decrease. The duration of breast-feeding is negatively correlated with microbiota maturation, delaying the maturation process (24), most likely due to the continuous domination by *Bifidobacterium*, *Lactobacillus*, *Collinsella*, *Megasphaera*, and *Veillonella* (26).

Around the age of 3-5 years, the microbiota has transitioned into a more adult-like ecosystem with a higher diversity (35,36). At this point, most of the species that will be present in adulthood are established, although relative abundance differs between children, adolescents, and adults. *Clostridium*, *B. longum*, *Bifidobacterium catenulatum* (37), *Faecalibacterium*, *Dialister*, *Roseburia*, and *Ruminococcus* (38) are some of the bacteria that are present at a higher abundance in children, while *Prevotella* and *Sutterella* are lower (37).

In adulthood, the microbiota reaches a relatively stable equilibrium dominated by Bacillota and Bacteroidota (39). Although a core microbiota might exist with microbial species present consistently across individuals, there is a large inter-individual variation depending on geography, genetics, and lifestyle (7,40). Despite this variation in composition, there is a functional redundancy, where a variety of species fulfill similar metabolic roles to maintain host homeostasis (39). Diet continues to be the main influence on the microbiota, although changes in diet often have a temporary effect, while antibiotics can change the microbiota up to at least 8 years post a single dose (41). Even though certain bacteria dominate the microbiota, it might be the presence of less abundant *Faecalibacterium prausnitzii*, *B. longum*, and *Bifidobacterium breve* that influence the stability of the gut microbiota in adults (40).

As age progresses, microbiota diversity often reduces, due to reduced variation in diet. This change in composition and function might contribute to immune dysregulation and systemic inflammation often seen in elderly people (19).

Understanding the dynamics and function of the gut microbiota is a crucial part in understanding how it contributes to the homeostasis of the host, both at the time of interaction and over a longer time.

1.1.3 Gut microbial metabolites

The gut microbiota has a large functional potential, including the capacity to degrade dietary components, xenobiotics, and host products into bioactive molecules. Within the gut environment, these metabolites maintain immunometabolism homeostasis by regulating energy metabolism and endocrine signals while balancing the proportions of mutualistic,

commensal, and pathogenic bacteria, as well as modulating mucosal gene expression and metabolic signaling pathways (10). While many microbial metabolites act locally, they also frequently cross the intestinal barrier to enter the host systemic circulation. This transit allows the gut to establish functional connections with distal organs, forming the gut-lung, gut-liver, and gut-brain axes (10,42).

Degradation and fermentation of dietary substances is a multi-step process and often involves several bacteria. This metabolic cross-feeding is a critical feature of the gut ecosystem in which the waste product of one species serve as the substrate for another (43,44).

One of the main bacterial metabolic processes in the intestine is the fermentation of complex carbohydrates, such as dietary fibers and HMOs into the SCFAs, butyrate, propionate, and acetate (45,46). As the microbiota develops and matures, the gut metabolite profile follows along, from low acetate and high succinate levels at birth to high acetate and formate levels at around one year and high propionate and butyrate when becoming two years of age (47). In the neonatal gut, the microbial metabolic landscape is dominated by butyrogenic bacteria, including *Lactobacillus* and *Bifidobacteria*, hydrolyzing HMOs from breast milk into SCFAs (48–52). The increase in butyrate at one year of age is correlated with the increase in *Clostridiales*, a known butyrate producing group (37,47). In certain cases, lactate is formed by fermentation, and while not being an energy source for the host, it is often cross-fed and further metabolized into SCFAs by bacteria such as *Eubacterium hallii* and *Veillonella* species (43,53,54). These SCFAs provide a vital energy source for the host and exert significant anti-inflammatory effects that strengthen intestinal barrier integrity (46).

Proteins are an important part of the human diet, providing essential amino acids, such as tryptophan, that cannot be synthesized by human cells. Tryptophan is metabolized in the gut by both bacteria and host cells into indoles. Bacterial species within, but not exclusive to the *Lactobacilli*, *Clostridium*, *Bacteroides*, *Peptostreptococcus*, and *Bifidobacterium* genera metabolize tryptophan into indole, indole-3-acetic acid, 3-indole acrylic acid, indole-3-aldehyde, indole-3 lactic acid, indole propionic acid, and tryptamine (55,56). The host utilizes two different tryptophan metabolic pathways, the kynurenine pathway and the serotonin pathway, regulated by tryptophan hydroxylases (Tph). The microbiota has been shown to influence the serotonin pathway by enhancing *Tph1* expression either directly or indirectly by SCFA and secondary bile acid interactions, as well as producing serotonin itself (57–59).

Sphingolipids (SLs) are a highly diverse class of lipids, primarily found in the cell membranes, lipoproteins, and other lipid-rich structures such as the skin of eukaryotic organisms, where they function as signaling molecules (60). They are mainly synthesized by the host and found in human breast milk (61). Further, SLs have other dietary sources (62), as well as being produced by Bacteroidetes (63–65).

1.2 The immune system

The neonatal immune system is not immature; it is a specialized developmental state, uniquely polarized to balance protection with tolerance.

— Inspired by from Kumar & Bhat and Angelone et al. (66,67)

The immune system is a network of cells, tissues, and soluble factors dedicated to maintaining homeostatic integrity. While traditionally viewed through the lens of host defense against pathogens, its primary daily function is surveillance and tolerance. By distinguishing between “self”, “harmless non-self” (such as nutrients and commensal bacteria), and “dangerous non-self”, the system protects the organism from infection, while actively preserving beneficial microbial communities.

The immune system is divided into two integrated arms, the innate and adaptive compartment. The innate immune system serves as the first line of defense, providing rapid but non-specific response through a combination of physical, chemical, and cellular barriers (68). In contrast, the adaptive immune system responds more slowly during an initial encounter but offers high antigen specificity and the capacity for long-term memory (68). Through the constant interaction of these two systems, the body achieves a sophisticated balance of immediate, broad protection and tailored, lasting defense.

1.2.1 The innate immune system

The primary defense against microbial invasion consists of physical protection, mediated by the epithelial layers of the skin and mucosal surfaces, which are reinforced by chemical deterrents such as acidic pH, AMPs, and lysozymes (69). When these barriers are breached, the innate immune system is mobilized. Central to this response are the myeloid cells, including monocytes, macrophages (M_{ϕ} s), dendritic cells (DCs), neutrophils, basophils, eosinophils, mast cells (MCs), and innate lymphoid cells (ILCs) such as natural killer (NK) cells (68). Monocytes, M_{ϕ} s, and DCs function as antigen-presenting cells (APCs), expressing a variety of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), NOD-like receptors, and C-type lectin receptors. They bind to pathogen- and damage-associated molecular patterns found at the site of infection (68). Activation of APCs leads to phagocytosis, followed by antigen presentation via major histocompatibility complex class II molecules (MHC II), as well as the release of cytokines and chemokines that recruit and activate additional immune cells. This combination of antigen presentation and recruitment of immune cells, is the critical bridge that initiates the adaptive immune response (68).

While most innate cells are of myeloid origin, ILCs represent a unique population of lymphoid-derived effectors that lack rearranged antigen-specific receptors. Instead of recognizing specific peptides as the adaptive lymphoid cells, ILCs respond rapidly to local cytokine signals from the epithelium, acting as early inducers of the innate immune

response. Today, four types of ILCs are identified: NK cells, ILC1, ILC2, and ILC3. While NK cells act in a cytotoxic and type 1 immunity way, the ILC1, ILC2, and ILC3 subset mirror the functionality of the CD4+ T helper (T_H) cells (T_{H1} , T_{H2} and T_{H17}/T_{H22} , respectively) and are enriched in tissue, particularly at barrier sites, such as the intestine and lung (70,71).

While immunological memory was traditionally attributed exclusively to the adaptive system (B- and T cells), recent evidence has highlighted the importance of “trained immunity” (72). This phenomenon involves the epigenetic and metabolic reprogramming of innate cells, including monocytes, ILCs, $M\phi$ s, and NK cells, as well as epithelial cells following exposure to microbial or foreign components (73). Intriguingly, emerging research suggests that these “trained” signatures may be transmissible to offspring through maternal exposure. This transgenerational priming potentially alters the immune status of the next generation, shaping their defensive landscape before they even encounter their first postnatal pathogen (74,75).

1.2.2 The adaptive immune system

While the innate immune system provides rapid, but limited specific clearance, adaptive immune cells are specialized to recognize and remember specific threats, mounting a targeted, though initially delayed, response. The system is primarily composed of T-lymphocytes (or T cells), B lymphocytes (or B cells), and the antibodies B cells produce. T cells originate from hematopoietic stem cells in the bone marrow but migrate to the thymus for maturation. Within the thymus, they differentiate into naive subtypes with either $\alpha\beta$ T-cell receptor (TCR) expression and becoming CD4+ helper or CD8+ cytotoxic T cells, or $\gamma\delta$ TCR expression. Through a process of random genetic rearrangement, an incredibly diverse repertoire of TCRs is generated. To ensure functionality and safety, these cells undergo a rigorous two-phase selection process. During positive selection, cells must demonstrate the ability to bind self-peptide-MHC complexes to receive survival signals, while negative selection eliminates cells that bind too strongly to self-peptides, either via apoptosis or by diverting to become regulatory T cells (T_{REG}) (68).

Mature naive T cells eventually migrate to the lymph nodes to encounter APCs. Activation requires a three-signal process involving the binding of the TCR to a specific peptide-MHC complex, the engagement of co-stimulatory ligands, such as CD28 on the T cell interacting with CD80 or CD86 on the APC, and a polarizing cytokines milieu. This triggers the expression of IL-2, driving clonal expansion and differentiation into armed effector T cells (76). While CD8+ cells become cytotoxic killer T cells that destroy infected host cells, CD4+ T helper (T_H) cells polarize into distinct lineages based on the local cytokine milieu (**Fig. 3**). Secretion of IL-12 by APCs induces the classical T_{H1} cells, whereas IL-4 induces T_{H2} cells. Beyond this traditional dichotomy (77), TGF- β and IL-6 will differentiate T_{H17} cells which will produce IL-17 to kill extracellular pathogens (78), and the presence of IL-6 and TNF- α initiates differentiation into T_{H22} cells, which produce IL-22 that helps maintain the integrity of epithelial barriers and secrete AMPs (79). Additional T cells have been

identified but are not mentioned in this thesis.

As hematopoietic stem cells travel to the bone marrow, they become B cells and develop their B cell receptor. For final activation, the B cells migrate to secondary lymphoid organs where they encounter soluble antigens which bind to the B cell receptor. Activated T_H cells interact with B cells by forming the CD40-CD40L complex, and alongside cytokine signals, this allows B cells to undergo immunoglobulin class switching. B cells can express five different classes of immunoglobulins (Ig): IgD, IgM, IgA, IgE, and IgG which all vary in structure, function, and distribution. As with T cell differentiation, immunoglobulin class switching is dictated by the cytokine environment. Th2 cytokines, including IL-4 and IL-13, preferentially drive class switching to IgG4 and IgE (80). This process creates a pool of memory B cells that respond rapidly during secondary encounters, forming a cornerstone of long-term adaptive immunity.

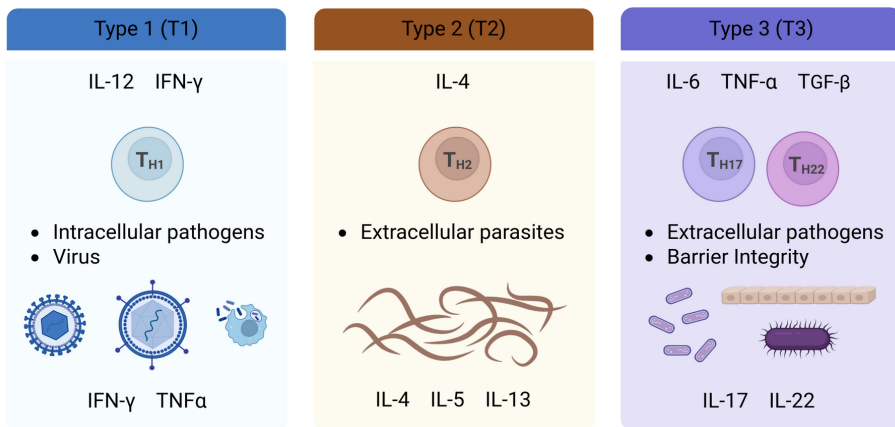


Figure 3: Immune types. Depicting the cytokines secreted by APCs that induce the different T_H cells, followed by their main immune function and the cytokines they secrete. *Created in Biorender.*

1.2.3 The ontogeny of immunity and the path to tolerance

Upon entering the world, the neonatal immune system is considered antigen-inexperienced, because newborns have not yet encountered environmental antigens, resulting in predominantly naive T and B cell repertoire. However, this system is not simply immature, it is uniquely regulated to balance protection and tolerance during early life.

The immune system in early life follows a characteristic developmental path (**Fig. 4**). Before birth, the fetal immune response leans toward an immune profile that helps limit strong inflammatory reactions and supports maternal-fetal tolerance. This tendency is reinforced by epigenetic patterns seen in cord blood T cells, where key T_{H1} -related genes are more heavily methylated, reducing their capacity to produce pro-inflammatory cytokines (66). After birth, this regulatory state lingers for a period, creating a generally low-inflammatory environment that supports the initial establishment of the infant's commensal microbiota.

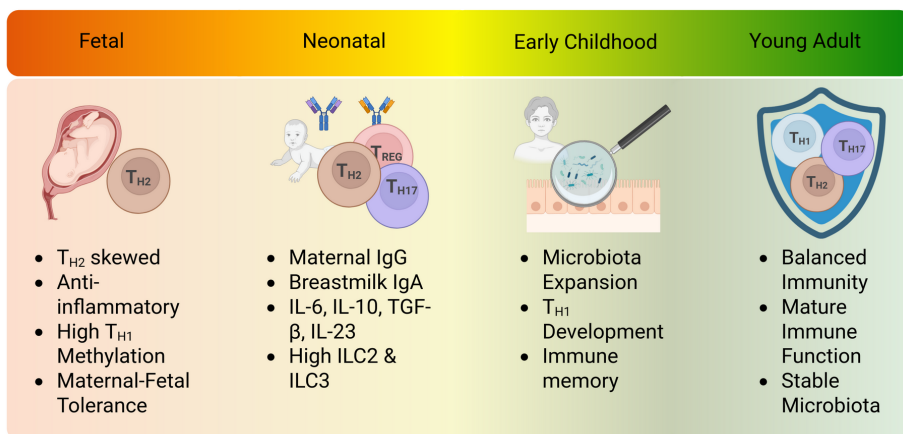


Figure 4: Ontogeny of the immune system. From fetal to young adult, how the immune system develops from TH2 skewed to a balanced system. *Created in Biorender.*

To compensate for this early immaturity, the newborn relies on passive immunity. Maternal IgG are transferred across the placenta in late pregnancy, and breast milk provides IgA together with cytokines and antimicrobial factors. These components not only protect against early infections but also help the initial shaping of the infant's microbiota (81).

In contrast, the infants' own innate defenses are often viewed as less efficient. As a result, neonates mount weaker responses to PRR activation. Early cytokines production is dominated by IL-6, IL-10, TGF-β, and IL-23 from M ϕ S and other APCs. This cytokine environment favors the development of T_{H2}, T_{H17}, and T_{REG} responses, while classic pro-inflammatory cytokines such as TNF-α and IL-1β remain low and rise only gradually over the first years of life (81,82). IL-6 also induces secretion of LPS-binding protein (LBP), a marker of intestinal permeability, indicating that the infant is sensing microbial products outside the GI tract even in this regulated environment (67,83).

ILCs are more abundant at birth compared to adulthood. ILC2s enhance the T_{H2}-skewed state of the newborn, supporting the mucosal homeostasis, but increasing susceptibility to allergic airway inflammation. ILC3s are vital for maintaining epithelial barrier integrity as the microbiota establishes itself (70,71).

Beyond conventional T cells, neonates possess specialized T cell populations like γδ T cells, MAIT cells, and CXCL8-producing T cells that provide early mucosal defense and IFN-γ, as counterbalance to the T_{H2}-dominant environment (82). Additionally, neonatal B cell immunity is prone to T_{H2}-type immune responses while the immunoglobulin repertoire is limited (82).

Overall, the neonatal immune system is not simply underdeveloped, it is carefully tuned for early life. By maintaining a T_{H2}-leaning, anti-inflammatory profile, infants avoid harmful systemic inflammation during the critical period of microbial colonization. While their innate

and adaptive responses are still maturing and rely heavily on maternal antibodies, specialized populations such as $\gamma\delta$ T cells and B-1 cells provide an important functional bridge, offering protection while preserving the tolerance needed for healthy immune and microbial development.

1.2.4 The young adult in balance

By the end of early childhood, the constant stimulation from the colonizing microbiota, repeated infections, and routine vaccination have assisted in driving the transition from a naive, tolerance-oriented state into a highly responsive and adaptable network. During this period, the adaptive immune system gradually builds a substantial immunological memory, allowing children to experience fewer infections and to mount quicker, more efficient responses when pathogens re-appear. The early T_{H2} -skewed profile seen in infancy slowly gives way to a more even equilibrium between Type 1 (T1), Type 2 (T2), and Type 3 (T3) immune pathways. This shift reflects the maturation of DC function, enhanced antigen presentation, a diversified TCR and B cell receptor repertoire, and the establishment of structured lymphoid tissues primed for rapid recall responses.

Microbial exposure continues to play a defining role during this stage. As the gut microbiota stabilizes its more adult-like configuration, it provides constant antigenic stimulation that promotes regulatory T cell development, reinforces oral tolerance, and strengthens epithelial barrier function. These interactions help calibrate the immune system toward a state in which inflammatory and regulatory pathways coexist in balance. The increasing production of pro-inflammatory cytokines such as TNF- α and IL-1 β , together with a more mature IL-12/IFN- γ axis, allows young adults to mount robust T1 responses against viruses and intracellular pathogens, something newborns are poorly equipped to do. Meanwhile, T2 and T3 pathways remain fully functional, but no longer dominate by default; instead, they are mobilized in response to helminths, allergens, fungi, and extracellular bacteria.

Crucially, the immune “programming” that occurs from birth through early childhood does not disappear; it sets out the foundation for adult immunity. A balanced young adult immune system is the result of successful early-life training: a well-regulated dialogue between the microbiota, epithelial barriers, innate sensors, and maturing T- and B-cell populations. When this developmental trajectory proceeds without major perturbation, the immune system reaches a homeostatic state in which tolerance to harmless antigens is maintained, inflammation is tightly controlled, and protective immunity is efficiently mobilized when needed. Conversely, deviations from this maturation pathway due to dysbiosis, infections, antibiotics, environmental exposures, or genetic susceptibility can shift the immune system towards the allergic, autoimmune, or inflammatory endotypes described later.

Thus, the young adult immune system represents the culmination of early-life learning: a balanced interplay of T1, T2, and T3 responses; a broad and functional memory pool; and a resilient mucosal interface shaped by years of microbial and environmental instruction. This

balanced immune state forms the reference point from which later-life immune dysregulation, including the trajectories seen in allergy and asthma, can be understood.

1.3 The barrier-microbe interface

*When the boundary fails, the seeds of
chronic disease are sown.*

— Based on Akdis (84)

1.3.1 Epithelial barrier in asthma

Recent research, including the Epithelial Barrier Hypothesis (84), suggests that many asthma endotypes originate with a failure of the body's very first line of defense: the airway epithelium. Under healthy conditions, the epithelial layer forms a tightly sealed barrier that keeps inhaled particles, microbes, and allergens at a safe distance from the underlying immune system. However, when this barrier is weakened, for example by air pollution, cigarette smoke, detergents, respiratory infections, or even a disturbed microbial community, the tight junctions loosen, and substances that should remain on the surface gain access to deeper tissues (85).

Once the barrier is breached, epithelial cells respond by releasing a family of alarm signals, or alarmins, most notably thymic stromal lymphopoietin (TSLP), IL-25, and IL-33. These molecules act like alarmins, alerting, and activating the immune system in a way that bridges environmental exposure with downstream inflammation. Critically, these alarmins do not dictate a single type of immune response. Instead, they set the stage for chronic activation across multiple pathways, fueling both T2-high and T2-low asthma endotypes (86). In T2-high disease, they drive eosinophilic inflammation through ILC2s and T_{H2} cells; in T2-low asthma, they promote neutrophilic or mixed inflammatory patterns through alternative innate and adaptive routes. In this sense, epithelial integrity, or the lack of it, becomes the common starting point for very different clinical outcomes. This perspective fits naturally with the earlier sections on immune development: the neonatal immune system depends heavily on the epithelial and mucosal barriers to regulate antigen exposure, and early microbial interactions help “train” these barriers to function properly. In healthy normal conditions, the young adult immune system reaches a balanced state where barrier function, tolerance, and controlled inflammation coexist, but when epithelial defenses are repeatedly damaged or never fully mature, the immune system is pushed toward chronic activation, setting the stage for asthma's diverse and persistent endotypes.

1.3.2 Colonic mucus system

The colonic mucus system is a vital component of mucosal immunity, functioning as both a physical barrier and an active immunological interface between the host and its dense microbial environment (87). The colon contains a two-layered mucus structure, in which the

IML is dense, firmly attached to the epithelium, and normally free of bacteria (88). Its primary role is to contain and spatially segregate the intestinal microbiota, preventing direct contact with epithelial cells while allowing controlled molecular exchange. This layer is continuously produced by goblet cells (GCs), specialized epithelial cells that secrete the glycoprotein MUC2, the main structural component of intestinal mucus (89). A subset of these cells known as sentinel GCs, can sense microbial breach or increased microbial activity through PRRs. Upon activation, they trigger a coordinated mucus-expulsion response that rapidly reinforces the IML and protects the underlying tissue. In addition to barrier maintenance, certain GCs also serve as GC-associated antigen passages, selectively sampling luminal material and delivering it to APCs in the lamina propria. Through this mechanism, GCs help shape tolerogenic immune responses to dietary antigens and commensal bacteria while limiting unnecessary inflammation. Notably, the function and maturation of the IML depend heavily on microbial relationship between the microbiota and the mucus barrier (90,91). Together, these features make the colonic mucus system a dynamic and highly regulated hub that integrates physical defense with immune education.

1.3.3 Gut associated host metabolites

Besides gut derived metabolites, the intestine contains many host and diet derived metabolites. While SCFAs are produced by fermentation in the gut, long chain fatty acids come from the dietary fats and adipose tissue. For the cells to use long chain fatty acids, these need to be transported into the mitochondria with the help of carnitines. As carnitines fuse with these fatty acids they form acylcarnitines (92). Carnitines are obtained through diet and mammalian synthesis and carnitine that does not get absorbed and reaches the colon will be used as carbon source by the bacteria (93).

1.4 Allergy and asthma

*Allergies are like your body's defense
system getting the wrong memo.*
– MacPhail (94)

Allergy and asthma are the result of hypersensitive reactions of the immune system towards non-harmful environmental substances, such as pollen, food, or animal dander. Asthma is one of the most common chronic non-communicable diseases worldwide, with airway related clinical symptoms including recurrent wheezing, breathlessness, chest tightness, and coughing. By 2025, more than 300 million people globally were estimated to be living with asthma, with the highest increases seen in rapidly urbanizing low- and middle-income countries (95,96). Despite improvements in inhaled therapies, the disease remains a major cause of preventable morbidity and mortality, particularly where treatment access is limited. This ongoing "asthma epidemic" highlights the need to move beyond symptom-based management toward a clearer understanding of the molecular pathways driving the

disease.

1.4.1 Immunological mechanisms of allergy

Allergic hypersensitive reactions can be categorized by inflammation and immune system-driven, tissue-driven mechanisms, or driven by direct chemical response, and in many cases one mechanism feeds into another. The inflammation and immune-driven category includes antibody-mediated and cell-mediated pathways. The antibody-mediated hypersensitivity types I-III operate through immediate, cytotoxic, or immune-complex mechanisms, and the cell-mediated reactions (type IVa-IVc) are classified according to their dominant T cell profiles: T1, T2, or T3. Tissue-driven mechanisms, in turn, reflect disturbances in the epithelial barrier (type V) or metabolic influences (type VI). The final category, type VII, represents reactions driven directly by chemicals (86). Type II, III and VII are not relevant for this thesis and therefore not further discussed.

Type I is a two-stages, IgE-dependent allergic response, initiated by typical allergens such as pollen, house dust mites, mold spores, animal molecule, food, drugs and much more. The first phase is the sensitization, in which the innate and adaptive immune system interact in a complex dynamic starting with the APCs encountering the allergen. After internalization of the allergen, the APC will initiate differentiation of naive T cells into T_{H2} cells. This is mainly done by DCs, but B cells and $M\phi$ s can also contribute to the differentiation. IL-4 is needed for the T2 direction and is secreted by MCs and basophils. The initial contact with the allergen will also trigger epithelial cells to release alarmins, such as IL-25, IL-33, and TSLP, activating ILC2s that then secrete IL-5, IL-9, IL-13. These T2 cytokines maintain the T2 differentiation of the T cells but also recruit eosinophils and induce mucus production in the lungs. The combination of IL-4, IL-13 and IL-21, derived from ILC2s and T follicular helper cells (T_{FH}) promote immunoglobulin class-switching on B cells, resulting in production of specific IgE (sIgE) with high affinity. These sIgE bind irreversibly to the high-affinity IgE receptor (FcεRI) on MCs and basophils, causing a strong reaction during secondary interaction with the allergen later in the effector phase. The initial sensitization phase is often symptomless, while in the effector phase the clinical allergic reaction occurs. FcεRI-expressing MCs and basophils encounter the allergen, a cross-linking of the receptors occurs, and the cells degranulate, releasing mediators including histamine, heparin, proteases, cytokines, prostaglandins, leukotrienes, and adenosine nucleotides. These mediators cause the classical symptoms of allergy (68,86). Clinical markers include elevated levels of fractional exhaled nitric oxide, serum IgE, and blood/sputum eosinophils (97).

Type IVb hypersensitivity represents the classical T_{H2} -driven allergic reaction, in which immune cells are the main mediators of the classical allergic reactions including airway inflammation in asthma. Basophils and NKT cells are common sources of the early IL-4 production and induce the differentiation of T_{H2} cells. These T_{H2} cells secrete large amounts of IL-4, IL-5, IL-9, IL-13, IL-31, as well as chemokines such as eotaxins that support eosinophil recruitment. As in type 1 hypersensitive, IL-4 and IL-13 drive IgE class-switching

in B cells, while IL-5 increases eosinophil activation and tissue infiltration. IL-13 additionally contributes to airway remodeling, a feature that characterizes the chronic inflammation with type IVb reactions and distinguishes them from the more acute type I responses. The inflammation is further reinforced by allergen-specific T_{H9} cells, whose production of IL-9 enhances IL-4-mediated IgE synthesis and supports the migration and survival of eosinophils and basophils, sustaining a T2-dominated inflammatory environment like what is seen in type I hypersensitivity (86).

Both type I and type IVb hypersensitivity are T2 skewed and result in IgE secretion. Type IVa hypersensitivity is instead a T1 endotype characterized by T_{H1} , ILC1, and NK cells which are activated upon allergen presentation by the APCs in combination with IL-12, IL-23, and IFN- γ . Activation causes a cascade of inflammatory reactions leading to tissue damage and clinical manifestation (86).

Type IVc hypersensitivity is initiated when APCs present their antigen in combination with IL-6, IL-21, IL-23, and TGF- β , after which T cells will differentiate into T_{H17} cells, and produce IL-17A, IL-17F, IL-21, IL-22 and granulocyte- $M\phi$ colony-stimulating factor. IL-17A and IL-17F normally protect against fungi and bacteria by promoting antimicrobial peptide, neutrophil recruitment, and enhanced epithelial barrier function, as well as activating ILC3 and stromal cells. ILC3 and stromal cells secrete in their turn IL-8, which recruits even more neutrophils causing local inflammation (86). This large engagement of neutrophils is the clinical diagnostic marker.

1.4.2 Asthma

Historically, asthma was categorized into clinical phenotypes such as early-onset allergic asthma, late-onset eosinophilic asthma, or obesity-related asthma. While clinically useful, these labels do not reflect the biological mechanisms responsible for the disease. Consequently, a shift toward endotype-based classification has emerged, grounded in the functional and immunological pathways underlying each presentation. Identifying a patient's endotype is now essential because it predicts the likelihood of responding to specific biological therapies (98).

The most basic division in asthma immunology is between T2-high and T2-low disease, but it is now clear that T1 and T3 immune pathways are also involved. T2-high asthma is the best-studied group and includes both allergic and non-allergic asthma (99). T2-low asthma, in contrast, is less understood and typically presents as either neutrophilic or paucigranulocytic disease (100,101).

Allergic asthma's main characteristic is the onset of airway hyperresponsiveness (AHR) by allergens in an IgE mediated manner. Allergic asthma often has its onset during childhood, as a culminating step of the atopic march. The diagnostic framework for asthma integrates clinical markers of inflammation with evidence of sensitization, commonly identified through skin prick testing and specific IgE assays (102).

Connecting the traditional term allergic asthma to the new nomenclature of allergic

diseases, it fits into both Type I and Type IVb as they both involve T2 immune pathways and result in the release of IgE (86).

Whether an early-life immune trajectory develops into a classic type-I, IgE-mediated allergic endotype or instead results in a type-IV to type-VI, tissue-linked or metabolic endotype depends heavily on the timing of the perturbation and the genetic predisposition of the host. In this view, asthma becomes not merely “episodic shortness of breath,” but a long-term immunological and metabolic imprint that is often established during the first months of life.

1.4.3 Genetic predisposition

One of the main risk factors for the development of allergy and asthma is family history. Having two parents with confirmed IgE mediated allergy increases the risk of having an allergy also in adult life compared to having healthy parents (103). Additionally, twin studies have shown that up to 70% of asthma susceptibility originates from genetic factors (104). This means that genetics plays an influential role in explaining asthma susceptibility (105). Genome-wide association studies have revealed a massive repertoire of genetic variants in asthmatics, although connecting these to development of the disease is not always easy. Many of the genomic modifications are found in non-coding gene regulatory elements, which control gene expression by binding of transcription factors. This interaction is highly influenced by epigenetic processes, such as DNA methylation and post-translational histone modifications (105).

Table 1: Genomic regions and the associated genes with SNPs associated to asthma.

Genomic location	Key genes
6p21.32	Many, including <i>MHC-I</i> and <i>-II</i>
1q21.3	<i>FLG</i> , <i>CRNN</i>
6p21.33	Many, including <i>MHC-I</i> and <i>-II</i>
10p14	<i>GATA3</i> , <i>SFTA1P</i>
5q31.1	<i>IL4</i> , <i>IL5</i> , <i>IL13</i>
11q13.5	<i>EMSY</i> , <i>LRRC32</i>
9p24.1	<i>IL33</i>
2q12.1	<i>IL1RL1</i> , <i>IL18R1</i> , <i>IL1RL2</i> , <i>IL18RAP</i> , <i>IL1R1</i>
5q22.1	<i>TSLP</i> , <i>WDR36</i>
10p15.1	<i>IL2RA</i>
17q21	<i>ORMDL3</i> , <i>GSDMB</i>

Many single nucleotide polymorphisms (SNPs) identified in asthma studies are found in the vicinity of T2 immune response genes, such as 2q12 (*IL1RL1*, encoding the IL-33 receptor), 5q22 (*TSLP*), 9p24 (*IL33*), 10p14 (*GATA3*), and 5q31 (*IL4-IL5-IL13*). Additional asthma inflammatory related genes with SNPs are *SMAD3*, *BACH2*, *TLR1/6/10*, and *STAT6*.

Strongly childhood asthma associated genes outside the immune response are *ORMDL3* and *GSDMB*, in the 17q21 locus, which are involved in cellular functions and apoptosis (**Table 1**) (106).

The evidence for a genetic connection decreases in adult-onset asthma compared to child-onset asthma, implying that environmental factors play a larger role over time (107).

1.5 Gut microbiota in health and disease

The relationship between genes and the environment can be compared to a loaded gun and its trigger. A loaded gun by itself causes no harm; it is only when the trigger is pulled that the potential for harm is released.

– Olden and Wilson (108)

Even though we have gained a deeper understanding of the pathophysiological mechanisms and the genetic background for many chronic diseases including asthma, these factors cannot fully explain the risk of falling sick. The collective exposure from the general external environment, specific external environment, and the internal environment plays a large role in disease development (109).

The gut microbiota is a central component of this internal environment, influencing the host while simultaneously being shaped by external inputs such as diet and antibiotics (110). For most of the host's life, this microbial community exists in a symbiotic balance, supplying energy through fermentation of dietary fibers, producing essential vitamins, and secreting bioactive compounds that maintain systemic homeostasis. The influence of these microbes extends far beyond the local intestinal environment; metabolites and bacteria can enter the systemic circulation or nervous system to reach distant organs, including the brain (111,112), liver (113), and lungs (114).

The input from the microbiota is especially important during childhood development (115). The first two to three years of life represent a “critical window” or “window of opportunity” for immune maturation. During this phase, the initial colonization of the GI tract serves as the primary driver of immunological health. This process is governed by stochastic assembly and priority effects, where the first colonizing species define the successional trajectory of the entire ecosystem (24,25,116). Any disruption during this period, such as reduced microbial diversity or dysbiosis, has been strongly correlated with a heightened risk of metabolic and immunological disorders, specifically childhood allergy and asthma (117–122).

1.5.1 Gut microbiota, allergy and asthma

The link between microbial exposure and atopy was first proposed through the Hygiene Hypothesis by Strachan (1989), suggesting that reduced exposure to childhood infections in cleaner, industrialized households increased the risk of eczema and hay fever (123). This theory has since evolved into the “Microflora Hypothesis”, focusing on the need for special

bacterial interactions, either from the environment or microbiota, to prime the immune system (124,125). While certain bacterial exposures are protective, others, such as early-life respiratory syncytial virus infections, have been shown to conversely increase asthma risk (126–128).

Several studies have shown correlations between altered microbiota compositions or delayed maturation of the gut microbiota with development of childhood allergies (129,130). Other studies have been able to pinpoint specific bacteria such as a group of Lactobacilli having a protective effect on allergy development during childhood (131,132).

The microbial influence on allergic diseases does not stop in childhood. Distinct differences are still observed in the microbiota of allergic adults. This raises a critical question regarding disease etiology: is this adult microbiota dysbiosis a driver of the ongoing disease, or merely a byproduct of chronic inflammation? It is also possible that these adult signatures are a trace of the early-life microbial disruption that originally tipped the immune system toward an allergic pathway (133,134).

1.5.2 Gut metabolites: the mechanistic link

The biochemical communication between the gut and the lungs, the gut-lung axis, is primarily mediated by microbial metabolites that function as systemic signaling molecules (135).

SCFAs, specifically acetate, propionate, and butyrate, are the most studied microbial metabolites in the context of immune regulation. Children with allergies or asthma have significantly lower levels of these fecal metabolites indicating that they play a role in disease development (117,136).

The physiological influence of SCFAs is achieved through two primary pathways: signaling via G protein-coupled receptors (GPCRs), such as GPR41 and GPR43, and the direct inhibition of histone deacetylases (HDACs) (46,137). GPCRs are widely expressed on epithelial cells, adipocytes, and immune cells including neutrophils, DCs, M ϕ s, and T cells. Activation of these receptors by SCFAs promotes recruitment of neutrophils to inflammatory sites and enhances the differentiation and suppressive capacity of T_{REG}s, which are thought to be essential in preventing allergic airway inflammation (138). Beyond receptor signaling, butyrate and propionate can diffuse into host cells to inhibit HDACs, thereby epigenetically altering gene expression toward an anti-inflammatory cellular phenotype.

The protective role of SCFAs also extends to the physical barrier by triggering mucin secretion to regulate mucosal homeostasis. In particular, butyrate is known to play a central role in maintaining intestinal mucosal immunity by supporting the barrier function of the gut and shaping local immune responses, partially by binding to aryl hydrocarbon receptors (AhR) on intestinal epithelial cells (139). It influences epithelial cell health and renewal while promoting balanced immune activity in the underlying tissue, helping limit inflammation and enhance antimicrobial defense. Furthermore, SCFAs stimulate the production of AMPs and fortify barrier integrity by up-regulating the expression of tight junction proteins like claudin

and occludin (140–142). Collectively, this highlights the importance of these SCFAs in the maintenance of gut mucosal homeostasis and links microbial metabolism to both innate and adaptive immunity.

A critical link between different metabolite classes exists through the AhR. Butyrate has been shown to induce AhR expression, effectively priming the sensor for other microbial signals. This receptor integrates environmental and microbial inputs, particularly tryptophan derivatives like indole-3-aldehyde, which serve as high-affinity AhR ligands. Activation of the AhR-mediated pathway triggers the transcription of several genes, including *IL-6*, *IL-22*, and *prostaglandin 2*, all of which are critical for maintaining gut barrier function and regulating intestinal immunity (56,143).

Finally, microbial influence on the host lipidome, specifically regarding SLs, has been implicated to play a role in asthma. Altered levels of SLs are a signature in asthmatic children (144) and can predict asthma exacerbation (145). While SLs are synthesized endogenously by the host, certain commensal bacteria within the *Bacteroides* and *Prevotella* genera possess the capacity to produce bacterial SLs (146). These microbial-derived SLs can inhibit host SL production (63) and are increasingly recognized as mediators in immune homeostasis in the gut and at distal mucosal sites.

1.6 Experimental models

*Mouse and human intestinal immunity: same ballpark,
different players; different rules, same score.*

— Gibbons & Spencer (147)

In clinical research, large-scale longitudinal cohorts have been the “eyes” of the field, allowing us to observe patterns, such as the consistent depletion of certain bacterial taxa in infants who later develop asthma (117,148). However, these clinical observations are inherently limited to correlation. In the human “black box”, it is nearly impossible to separate whether a microbial shift is the cause of the asthma, a result of the environment, or a byproduct of the inflammation itself.

To move from correlation to causation, we must take these clinical “signatures” into *in vivo* models. Mouse models, particularly germ-free (GF) mice, allow us to test the hypothesis that the microbiota is the driver of the disease. By colonizing a sterile host with specific “asthma-associated” communities, we can observe the direct impact on immune maturation and airway hyper-responsiveness. This stage of the pipeline is where we prove that the “loaded gun” of the environment actually pulls the “trigger” of the immune system.

Finally, to understand the exact molecular language being used, we must deconstruct the system further into bioreactors. While the mouse shows us the effect, the bioreactor shows us the mechanism. These controlled fermentation systems allow us to isolate the bacteria from the host and measure their metabolic output with high precision. It is here that we can determine exactly which fibers lead to the production of protective SCFAs or how a specific

pH change might halt the production of tryptophan-derived AhR ligands. By combining the complexity of the mouse and the controlled environment of the bioreactor, we can finally define the “active ingredients” of a healthy microbiota.

1.6.1 Human studies

Human clinical studies have long been the primary source of our understanding of the microbiota-asthma connection. Historically, these studies relied on stool samples, which offer a non-invasive but limited “snapshot” of the distal colon’s end-products. However, as our understanding of the gut-lung axis matures, it has become clear that immune education and metabolite production occur throughout the GI tract.

In recent years, small devices for *in vivo* spatial mapping have been developed to give us a better view of the microbiota and its environment. The SmartPill can measure real-time parameters such as segmental pH and transit time of different parts of the GI tract, providing us with another dimension of understanding of factors directly dictating the metabolic behavior of the microbiota (15,16). Another innovation is CapScan, an intestinal sampling device that can sample the gut microbiota and its metabolites at defined locations in the intestine depending on pH (15,149). These devices give us a deeper insight in what is happening inside the gut but they are still limited to “snapshots”.

Human clinical research is frequently restricted to observational data, particularly when considering potentially harmful interventions that would be ethically unfeasible to test in humans. Furthermore, the immense variety of uncontrollable environmental variables, ranging from diet and pollution to household size, and hereditary input creates a high level of noise. Accounting for these co-variables necessitates exceptionally large sample sizes to achieve the statistical power required for identifying clear, reproducible outcomes. While these studies provide the essential real-world context for asthma research, they remain limited by their inability to definitively separate cause from effect.

1.6.2 Mouse models

To bridge the gap between clinical patterns and biological proof, we have to move into controlled experimental systems. The mouse offers an *in vivo* possibility free from many environmental and genetic variations that are impossible to eliminate in human studies. Mice allow for high-risk intervention studies and the strict regulation of environmental variables that are impossible to manage in human cohorts.

The use of GF mice has been fundamental in demonstrating that the maturation of the immune system (150) and physical development of the GI tract are dependent on microbial inputs (91,151). By using HMA mice, where GF mice receive a human fecal microbial transplant (FMT), research has shown us the effective transfer of a human disease phenotype into mice (152,153). This approach allows for the deconstruction of complex human microbial signatures into smaller subsets or even monocolonizations with single strains (154,155). Such models have successfully identified key immunomodulator taxa; for

instance, segmented filamentous bacteria and *Bifidobacterium adolescentis* have been shown to specifically induce T_{H17} cell populations (156,157).

However, the transition from human to mouse is rarely seamless. The engraftment of human microbiota into a murine host is never 100% successful. Several species exhibit host-specificity (159), and colonization success is heavily influenced by the inoculation method (118,160), timing (161), and the specific diet of the mouse (162). If a key symbiont fails to colonize the mouse, its specific metabolic contributions, such as specific SCFA profiles or lipid signatures, may be missed.

Furthermore, the clinical relevance of mouse models is often challenged by fundamental biological differences in the murine immune system compared to humans (147). These translational gaps must be carefully considered when interpreting how a mechanism discovered in a mouse might behave in a human patient.

1.6.3 Bioreactors

Human and mouse studies have taught us a great deal about how microbes interact with their hosts, but they often blur the view of the microbes themselves. To understand the microbiota on its own terms, we should study it as an independent ecological system. *In vitro* bioreactors allow us to study the dynamic interactions, competition, and metabolic shifts that occur within the microbial population. By isolating the microbiota from the host, we can see how the community reorganizes in response to environmental changes and identify which metabolic products are a direct result of microbial activity. This level of isolation is essential for defining the true functional potential of the gut microbiota.

A major strength of bioreactors is the precision with which the environment can be controlled. In a bioreactor, we can strictly control the atmospheric conditions, mimicking the anaerobic environment of the GI tract, by using either nitrogen or carbon dioxide (164) gases or letting the bacterial fermentation (165) taking care of any oxygen exclusion. Nutrient inputs can be tuned just as carefully. This level of control extends to the diet, where we can alter the growth medium and we can observe how specific species with distinct nutritional requirements react to different substrates, as well as studying the metabolic output. This makes it possible to evaluate prebiotic effects of dietary fibers in isolation and identifying exactly which fibers shift the metabolic output toward anti-inflammatory profiles (166).

Unlike the "snapshot" nature of clinical stool samples, dynamic gut models provide a continuous, longitudinal view of microbial behavior. Modern systems, such as the TIM-1/2, the PolyFermS or the SHIME, have proven to be highly reproducible and capable of mimicking the distinct environments of either parts of the GI tract, such as the ileum (167,168) and the various regions of the colon (169,170), or the entire GI tract from stomach to descending colon (171). These models have shown that we can successfully cultivate communities that closely resemble the human microbiota, providing a method to study the microbial dynamics of the gut.

Finally, bioreactors offer a safe, controlled platform for perturbation experiments.

Antibiotics, toxins, or other stressors such as pathogens or antibiotic-resistant bacteria can be introduced without risk to a host, allowing research on the microbial community resilience, collapse or reorganization. With the immune system removed from the equation, the bioreactor allows us to study the microbiota and metabolic flux in a way that is not possible *in vivo*, giving us the underlying microbial biochemical mechanisms and ecological connections that result in the metabolites priming the immune system.

2 Research aim

The **overall aim** of this thesis was to characterize the composition of the gut microbiota across various experimental settings, and to evaluate its potential to influence host health, specifically in the context of allergy and asthma.

Specific aims:

- To determine whether the presence of allergic asthma at age 20 can be traced back to specific immune and gut microbiota signatures present during early life. (**Paper I**)
- To investigate the colonization patterns of human infant microbiota from donors with varying allergic risk, within a murine model and to assess their subsequent influence on the host metabolome. (**Paper II**)
- To design and validate an anaerobic bioreactor capable of culturing a complex, gut-like microbiota to investigate the effects of antibiotic perturbation. (**Paper III**)

3 Materials and methods

The following section provides a summary of, and reflection over the methodology and materials used throughout my research. For more details regarding specific experiments, please refer to the individual papers.

3.1 Cohorts and other human material

For the work in this thesis, samples collected from the BIAS (Barn Immunologi Allergi Sachsska) study were used.

The BIAS study is a longitudinal birth cohort consisting of 281 children born in Stockholm between 1997 and 2000. Enrollment took place at Sachs' Children and Youth Hospital prior to birth. Ethical clearance was granted by the Human Ethics Committee at Huddinge University Hospital, Stockholm (Dnr 75/97, 113/97, 331/02, 2007/858-31/2, 2019-02034); parental consent was obtained verbally at the start of the study, followed by written consent from the participants upon reaching age 20.

Parental history of allergy was verified through medical history and confirmed via skin prick tests or sIgE measurements. Based on these findings, participants were sorted into three hereditary categories: neither parent was allergic, (No Heredity, NH), only the mother had allergies (Single Heredity, SH), or both parents were allergic (Double Heredity, DH).

Clinical assessments focused on the onset of allergic conditions were conducted at multiple intervals, including 6, 12, and 18 months, as well as at 2, 5, 10, and 20 years. Blood samples were collected at 6 and 12 months, and at 2, 5, 10, and 20 years, while fecal samples were available from 1, 2, and 3 weeks, 1, 2, 3, 6, 12, 18 months, and at 2 years (132,172–175).

Material from this cohort was used for both paper I and paper II. **Paper I** utilized a subset of the DH group, selected based on available 20-year peripheral blood sample and at least one fecal sample from the participant's first month of life. For analysis, these individuals were stratified by their asthma status at age 20. In **paper II**, fecal samples from a subset of individuals were used to inoculate GF mice.

Additionally, **paper II** also involved peripheral blood mononuclear cells (PBMCs) from anonymous adult donors, which did not require specific ethical permit as the donors remained unidentifiable.

3.2 Paper I

In this study, we analyzed a subset of 26 participants from the BIAS cohort characterized by DH. Inclusion was based on the availability of both neonatal fecal samples and 20-year follow-up blood samples. These were supplemented by maternal and intermediate samples (years 1 and 2) when available. Participants were grouped into asthmatic ($n = 12$) and non-asthmatic ($n = 14$) groups based on clinical symptoms and IgE sensitization to common allergens at the 20-year follow-up.

PBMCs, isolated at ages 2 and 20 years and stored in liquid nitrogen, were processed via flow cytometry to quantify T cell-, DC-, and monocyte populations. RNA sequencing was performed on these samples to assess differential gene expression and functional enrichment. Plasma cytokines and chemokine levels were quantified using the Olink platform, utilizing Normalized Protein eXpression (NPX) values.

To characterize the early-life microbiota, profiles were generated from fecal samples (1 week, 4 weeks, 1 year, and 2 years) using 16S rRNA sequencing of the V3-V4 regions. Bioinformatic analysis included alpha and beta diversity metrics, differential abundance testing, and PICRUSt2 functional predictions. Statistical significance was determined using non-parametric tests (paired or unpaired as appropriate), with corrections for multiple testing where applicable.

3.3 Paper II

Fecal matter from infants from the BIAS study was used to create a two-generation HMA mouse model and to stimulate human PBMCs. Fecal samples from 6-7 infants (aged 2-8 weeks) were pooled based on parental allergic heredity and allergic clinical outcome at age 2:

- Non-allergy-associated microbiota (non-AAM): Pooled feces from children with DH who remained healthy at age 2.
- Allergy-associated microbiota (AAM): Pooled feces from children with DH who developed allergies by age 2.
- Control: Pooled feces from children with NH who remained non-allergic at age 2.

Adult PBMCs were stimulated with fecal water prepared from non-AAM and AAM samples. Cytokine production was measured via ELISA, and fecal water content was analyzed by LC-MS/MS.

For the animal model, infant fecal pools were transplanted into GF dams via gavage. Following microbiota stabilization, dams were bred and received a booster gavage prior to delivery. Dams were sacrificed four weeks postpartum for cecal content collection. Offspring were sacrificed at 6 weeks of age; samples collected included cecum and large intestine content, liver, intestinal tissue, abdominal fat and plasma.

Microbial DNA from the intestinal content and human infant fecal samples was extracted for 16S rRNA amplicon sequencing (V3-V4 region). Mouse organs and plasma were analyzed via LC-MS/MS for sphingolipids and polar metabolites, while SCFAs in the liver and cecum tissue were quantified by GC-MS.

Additional fecal samples from six GF mice from the same facility were analyzed for their microbial content by 16S rRNA amplicon sequencing.

Sequence data were processed using DADA2 and SILVA database (v138.1), with all samples rarefied to 5,570 reads (**Paper II, Supplementary figure 2**). For analysis, dam fecal and cecal samples were grouped as “dam”, while large intestine and cecal samples

from offspring were grouped as “offspring” due to compositional similarities.

3.4 Paper III

The objective of this study was to design and validate a cost-efficient anaerobic bioreactor system for the cultivation of gut-derived bacterial communities. The experimental validation was conducted in two runs (experiment 1 and 2).

System design and environmental control

The culture vessels consisted of modified 1-liter Schott Duran glass bottles (**Paper III, Fig. 1**). In addition to the standard GL45 neck, each vessel was customized with two vertical GL14 sampling ports and two angled GL25 ports. The GL45 central cap was equipped with a drive shaft connected to a magnetic propeller for agitation. Environmental parameters were regulated via the FerMac20 system (Electrolab Biotech).

Each vessel contained 700 mL of culture media maintained under continuous agitation at 200 rpm. Temperature was stabilized at 37°C using heating jackets and a thermometer inserted through a GL14 port, connected to the FerMac 240 temperature controller. A Fermac 260 pH controller, connected to a pH probe in a GL25 port, maintained a pH range of 6.4-6.8 via the automated addition of 0.05 M HCl or 0.5 M NaHCO₃. To maintain anaerobic conditions, reactors were intermittently flushed with sterile CO₂ or N₂ gas. L-cysteine was used as a reducing agent, and media was autoclaved prior to the experiment to ensure an anaerobic environment.

Inoculation and experimental design

Human fecal samples were obtained from a healthy donor and were collected at two separate time points. Fecal suspensions (10% w/v) were prepared under anaerobic conditions. Experiment 1 used fresh fecal slurry and supernatant, while experiment 2 was inoculated with glycerol preserved stocks to improve inter-run reproducibility.

Six media formulations (**Paper III, Supplementary table 1**) were initially screened in a batch setup to identify the optimal composition for growth. Community composition was evaluated via 16S rRNA sequencing.

For the bioreactor experiments, the vessels were stabilized for 18 hours post-sterilization and montage, before inoculation. A steady state was monitored for 20 days; during which the gas source was shifted from CO₂ to N₂ at day 9 to assess the impact of redox changes on the community. On day 21, three reactors were treated with a single dose of metronidazole (29.674 μg mL⁻¹), while one reactor served as a PBS-injected control. Samples were collected hourly during the first six hours post-treatment, followed by sampling at 6, 12, and 24 hours intervals.

Bioinformatics and statistical analysis

Microbial community dynamics were analyzed retroactively using high-throughput

sequencing. DNA was extracted for 16S rRNA (V3-V4) sequencing on the Illumina MiSeq platform. Sequences were processed through the DADA2 pipeline for quality filtering and amplicon sequence variant (ASV) identification using the SILVA database (v132). Alpha and beta diversity metrics were calculated using the phyloseq and vegan packages in R. Significant shifts in community structure were determined via PERMANOVA, and synergistic bacterial networks were identified through Multi-Dimensional Feature Selection (MDFS).

3.5 Methodological considerations

General analytical considerations: 16S rRNA vs. metagenomics

In all three papers, bacterial community profiling via 16S rRNA sequencing was the primary analytical method. While metagenomic shotgun sequencing would have provided higher taxonomic resolution and insights into functional genomic potential, 16S rRNA was selected to balance cost-efficiency with high sampling frequency. This approach allowed for the longitudinal tracking of community dynamics across multiple time points and conditions, which was central to the aims of this thesis.

Paper I

A primary challenge in **Paper I** was the limited sample size in combination with the longitudinal nature of the BIAS cohort. Due to the previous studies performed on this cohort, complete sets (fecal, plasma, and blood from all time-points) were not available for all participants. This reduced statistical power and constrained certain correlation analysis.

The long-term storage of blood and fecal samples presented specific technical hurdles. Specifically, the 2-year PBMC samples had been stored in liquid nitrogen for over two decades, which sometimes significantly impacted cell viability. While initial stimulation experiments were attempted, the resulting data were occasionally unreliable and were excluded from the final analysis. Still, samples from both groups had an equal storage time and it is unlikely that storage time influenced one group more than the other. Accordingly, we have focused on differences between groups rather than exact numbers and percentages within one group. Furthermore, long-term storage of plasma at -80°C may have resulted in freeze-drying, potentially inflating protein concentrations. To mitigate this, we calculated the ratio between 20-year and 2-year levels, assuming that concentration changes affected both clinical groups equally.

Regarding the fecal samples, DNA extraction remained effective despite low starting material (< 25 mg) in several samples.

Paper II

The mouse model used in **paper II** was initially created to identify immunological differences in offspring based on the origin of the microbiota (153). Fecal matter from several infants with similar genetic and clinical backgrounds was pooled for inoculation of 3-4 dams per group. While pooling communities is common in HMA models, it is debatable practice

from both microbiological and statistical perspectives. Microbiologically, pooling distinct communities creates a new dynamic that requires stabilization, introducing an element of stochasticity into the model. Statistically, inoculating 3-4 dams with a single pooled sample creates experimental replicates rather than true biological replicates. However, this design was counterbalanced by using pooled material that represents several biological donors (170), thereby capturing a broader range of the target phenotype's microbial diversity. A significant strength of this study was the inclusion of metabolomic analysis of intestinal content and fecal water. This allowed us to confirm functional transitions and metabolic activity despite the compositional shifts that inevitably occur after human-to-mouse transplantation.

Finally, due to the exploratory and observational nature of the metabolomic data, differential abundance was tested without FDR adjustment to avoid the omission of potentially relevant biological signals.

Paper III

The development of the bioreactor system involved several critical design iterations regarding media composition, anaerobic conditions, and inoculation strategy.

While many systems use complex media (e.g., Macfarlane et al. (177)), the large volume of our vessels made this cost-ineffective. We developed a simple medium that, through iterative testing supported a bacterial community more representative of the original fecal samples. While further optimization could target fastidious taxa, the current composition proved sufficient for modeling general community dynamics.

As the bioreactors were operated in an aerobic environment, ensuring an anaerobic environment inside was vital. Although silicone tubing is somewhat oxygen-permeable, the use of L-cysteine as a reducing agent and the inclusion of resazurin as a visual oxygen indicator confirmed that the system remained functionally anaerobic. While the oxygen sensors initially installed were not sensitive enough for the microaerobic threshold required, the stability of the strictly anaerobic taxa in our sequencing data retroactively validated the integrity.

Transitioning from fresh fecal slurries and supernatants (Experiment 1) to glycerol stocks (Experiment 2) was a key improvement for future reproducibility. Furthermore, we observed that intermittent CO₂ flushing caused transient pH drops due to the formation of carbonic acid. Switching to N₂ at day 9 resulted in a more stable microbial community. While a constant flow of gas would have been optimal to maintain continuous overpressure, our intermittent flushing protocol was sufficient to maintain the desired anaerobic environment.

The metronidazole challenge was calculated to simulate a single dose distributed throughout the total body weight of an average woman. We acknowledge that this is a simplification; in a clinical setting, an oral dose would result in significantly higher concentrations within the gut. This limitation should be considered when interpreting the magnitude of the community shift following treatment.

4 Results and Discussion

4.1 Paper I - Asthma in young adults at high risk for allergies is traced back to immune- and microbiota signatures in early childhood.

In this longitudinal sub-study we selected 26 individuals with DH for allergy with samples available from infancy and at 20 years of age to determine whether early-life immune and gut microbiota features could be associated with the development of allergic asthma in young adulthood. Twelve participants developed allergic asthma by age 20, while fourteen did not. Although both groups shared similar early-life environments and genetic risk, we observed several immunological and microbial differences early in life that distinguished future asthmatics from non-asthmatics.

At 20 years, PBMC phenotyping revealed no major differences in T-cell subsets or DC activation markers between groups. This was most likely due to shared genetic predisposition and the immunomodulatory effects of asthma medication. However, a trend toward higher FcεRI expression on DCs was observed in asthmatics.

In contrast, at 2 years of age, we observed clear innate immune differences. Future asthmatics showed a significant higher frequency of FcεRI⁺ DCs and a significantly lower frequency of PD-L1⁺ DCs. While a lower PD-L1 expression on DCs is clearly associated with a reduced regulatory phenotype (178), the role of FcεRI on DCs is less clear. Although some studies implicate a role of this receptor in tolerance, there is also data to support enhanced antigen uptake and presentation of allergens to T-cells, in particularly when IgE levels are high (179,180). T-cell frequencies remained similar, indicating that early innate immune deviations, and not adaptive immunity, were more predictive of later asthma. However, it is possible that functional adaptive responses would have been different, also in relation to the differences seen on the DCs, but this was not further investigated in this study due to limited material and cells with long storage time which are not suitable for functional analyses.

Transcriptomic analyses reinforced and expanded this pattern. At age 20, 60 differentially expressed genes (DEGs) distinguished asthmatics from non-asthmatics, including down regulation of asthma-associated genes such as *HRH4* (181,182), *SERPINB2* (183), and *PTGS2* (184). Pathway analysis showed suppressed responses to bacterial and inflammatory stimuli and increased RNA-processing pathways.

At age 2, transcriptomic differences were even more pronounced, with 216 DEGs. Several genes previously linked to allergy or asthma were altered, including up regulation of *DUSP8*, involved in T_{H9} development and promoting asthma and atopic dermatitis in mice (185), and *FFAR1* and down regulation of *TNFAIP8L2* (186,187). We once again found more differences between the groups at 2 years than at 20 years, suggesting that early immune dysregulation precedes clinical disease. Pathways related to cytokines signaling viral response, and general immune activation were suppressed, while RNA-processing pathways were enriched, an emerging theme in asthma biology (188,189).

The differences observed in the pathway analysis at both 2 and 20 years indicate a more general immune dysregulation in the asthmatic group, which could be an early indicator of later development of the disease. This idea is supported by previous research showing signs of impairment of innate immunity and overall T1 responses in allergic asthma (190).

Plasma cytokines and chemokines levels did not differ significantly between groups at either age, likely reflecting strong genetic influences on circulating protein concentrations (191,192). Some trends emerged when we looked at 20- relative to 2 years ratio with higher IL-33, TNF, and IL-13 ratios in asthmatics but none reached significance. Barrier-related proteins also showed no major difference, though uteroglobin tended to be lower in asthmatics in both ages, consistent with prior asthma studies (193). No differences in LBP were observed between the asthmatic and control group at neither 2 or 20 years. Although, when we tested levels of LBP in non-allergic individuals with no allergic parents (NH) from the same cohort, we observed higher levels of LBP in the DH individuals compared to the NH at 2 years. At 20 the LBP concentration in the NH group had increased to similar levels as the DH groups (Fig. 5). This could indicate that both groups with DH have higher gut epithelial permeability due to their genetic background, making the composition of the gut microbiota more important as they have increased possibilities for interactions with the immune system.

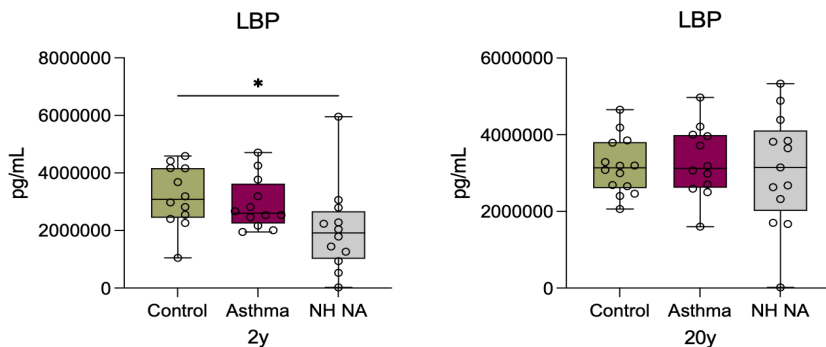


Figure 5: LBP in plasma at 2 and 20 years. LBP concentrations in plasma for the individuals with DH that did not develop asthma at 20 years (control), the individuals with DH and developed asthma at 20y (asthma), as well as the non-hereditary, not allergic group.

Gut microbiota analysis from birth to age 2 revealed dynamic developmental patterns in both groups, with diversity increasing over time. While α - and β -diversity did not differ significantly between the groups, specific taxa and temporal trajectories did. This is expected between the age of 1 week and 2 years as the microbiota is in its major developmental stage and highly influenced by the changing diet and environment.

Differences in developmental patterns between the groups were seen in the relative abundance of *Bifidobacterium*, which was higher in non-asthmatics at 1 week and 2 years,

but from 4 weeks to 1 year the opposite was true. *Bifidobacterium* is renowned for promoting gut health and reducing the risk of allergic disease, including asthma (194,195). However, its abundance naturally diminishes over time, and the genus is heterogeneous, with species-specific metabolic functions affecting the host (196). Therefore, while we could not investigate species-level differences between groups, we hypothesize that the presence of certain *Bifidobacteria*, at defined abundances and time points, contributed to protection in the non-asthmatic group.

Interestingly, asthmatics showed higher levels of *Escherichia-Shigella*, *Enterococcus*, and *Erysipelatoclostridium* at the early time points, which all have been associated with either childhood asthma or allergy (197–200). Additionally, several taxa previously seen at lower levels in individuals with asthma (148,201,202), including *Bacteroides* and *Ruminococcus*, were reduced in future asthmatics during the 2 years of development.

Delivery mode partially overlapped with these patterns, as more asthmatics were born by c-section, but this could not account for the remaining cases. Interestingly, α -diversity in the children correlated with α -diversity in their mothers, and about 20% of the maternal fecal microbiota was recovered in the corresponding child. Together this indicates that differences seen in the asthmatics was not due to delivery mode, but due to initial higher α -diversity in their mothers contributed to vertical transmission (203,204).

Functional predictions using PICRUST2 revealed that at 1-week, future asthmatics had higher potential for purine and pyrimidine degradation pathways, metabolic signatures also seen in murine asthma models. At 2 years, non-asthmatics showed higher predicted capacity for SCFA production, including pathways linked to the *Bifidobacterium* shunt and TCA cycle. Given the known protective effects of SCFAs, these findings support a mechanistic link between early microbial metabolism and long-term asthma risk.

This study provides rare long-term evidence that early-life immune and gut microbiota signatures are associated with allergic asthma development in young adulthood, even among individuals with uniformly high genetic risk.

4.2 Paper II - Impact of early-life human microbiota on the murine host metabolome: insights from a two-generation HMA mouse model and implications for allergic disease.

While we in paper I studied how the early-life microbiota potential primes the immune system for allergic asthma development, in paper II, we investigated how the microbiota of a subset of these children affects peripheral immune response *in vitro*. This initial experiment was complemented by inoculation of GF mice with the same fecal samples to study potential differences in microbiota trajectories and host metabolome related to different microbiota composition in the inoculate. We used a two-generational HMA mouse model in which the dams got the human FMT and the offspring was metabolically profiled, to capture the true early-influence. Additionally, we followed the trajectory of the different microbiota compositions into the mice, to confirm colonization and clinical relevance of the model. By

combining *in vitro* stimulation of human immune cells with an *in vivo* two-generation HMA mouse model, paper II bridges observational human data with mechanistic experimental insights.

The metabolite composition of the infant fecal pools differed clearly between the allergy-associated (AAM) and non-allergy-associated (non-AAM) groups. The non-AAM samples contained higher levels of several tryptophan-derived compounds, such as tryptamine and indole-lactic acid, whereas the AAM samples were enriched in long-chain acylcarnitines. These differences suggest that the two microbial communities are functionally distinct, resulting in different metabolic profiles. In particular, the enrichment of tryptophan-derived metabolites in the non-AAM group is interesting given their influence on immune regulation and epithelial homeostasis (56,143), whereas elevated acylcarnitines have previously been linked to altered lipid metabolism and inflammatory states (205).

These metabolic differences translated into divergent immune responses when human PBMCs were stimulated with the fecal waters. AAM-derived material triggered strong T1, T2, and T3 cytokines production, while non-AAM fecal water induced a more regulatory innate profile dominated by IL-6 and IL-10 (**Paper II, Fig. 2**). This suggests that early-life microbial metabolites can shape immune activation patterns in ways relevant to allergy risk. Interestingly, a previous study from our group phenotyped the same HMA mice used in this study and saw that cells derived from the intestine and spleen of these mice showed a distinct T3 immune profile (153). Indicating that priming of the immune system with the AAM induces a persistent T3 profile that is detectable *ex-vivo*. The findings in the two setups, the *in vitro* PBMC stimulation and *in vivo* immune profile analysis, strengthens the conclusion that early microbial exposure leads to long lasting immune imprinting.

Once introduced into GF mice, the human microbiota adapted to the murine environment as expected (152) but retained recognizable group-specific signatures. Interestingly, all three groups showed a reduction in *Bifidobacterium* and an increase in *Bacteroides*. This could be explained by the shift in diet from breast-fed to chow. Although the microbial communities showed similar shifts after colonization, each inoculum produced a distinct and stable trajectory in both dams and offspring, demonstrating that early-life human microbial differences persist even after cross-species transfer and adaptation.

Offspring microbiota resembled their respective dams more than other groups, indicating successful vertical transmission and stabilization across generations. Certain taxa, such as *Hungatella*, *Enterobacter*, *Clostridiodes*, and *Collinsella*, were particularly associated with the AAM trajectory, while the non-AAM and control group were driven by *Bifidobacterium*, *Lactacaseibacillus*, and *Enterococcus*.

Across multiple compartments, intestinal content, intestinal tissue, liver, adipose tissue, and serum, the metabolic profiles of the offspring showed clear group-dependent patterns. SLs, acylcarnitines, and tryptophan metabolites were especially informative. Importantly, the metabolic patterns in the mouse offspring mirrored those found in the corresponding human infant fecal waters with elevated levels of essential amino acids and tryptophan-derivates in

the non-AAM mice. This cross-species consistency strengthens the idea that early-life microbial communities influence the host physiology by their metabolic production and that the effect can be preserved across different host environments.

Overall, our findings show that early-life microbiota composition is a strong determinant of host metabolic programming and that the two-generation HMA model effectively captures human-relevant microbiota-metabolome interactions. The divergence between AAM and non-AAM groups, both in microbial composition and in downstream metabolic and immune-modulating outputs, suggests that allergy risk is shaped by complex functional networks rather than isolated taxa. While species-specific differences between mice and humans must be considered, the resemblance of the results across the *in vitro* human system and the *in vivo* mouse model supports the functional robustness of the microbiota and this model.

This study therefore provides mechanistic support for the idea that early microbial ecosystems influence later immune trajectories through metabolome-level effects, reinforcing the importance of early-life microbial development in allergy research.

4.3 Paper III - Establishment of Human Gut Microbiota into Bioreactors shows a clear Niche Adaptation.

In my last study we demonstrated that our *in vitro* bioreactors could sustain a substantial portion of the human gut microbiota and reproduce key ecological dynamics under controlled conditions. Across two independent experiments, the reactors preserved roughly 85% of the α -diversity of the original fecal inoculum and reached a stable community structure within the first days of operation.

Initial batch experiments comparing six media formulations showed that all media altered the microbial composition, favoring fast-growing, metabolically flexible taxa such as *Bifidobacteriaceae* and *Enterobacteriaceae*, while more fastidious families, including *Ruminococcaceae*, *Veillonellaceae*, *Ruminococcaceae*, and *Lachnospiraceae*, declined. Mucin is an important carbohydrate source for several bacteria, including *Akkermansia muciniphila*, *Ruminococcus torques*, and members of the *Lachnospiraceae* family (206). Although mucin supplementation increased *Bifidobacteriaceae*, neither *Akkermansiaceae* or *Ruminococcaceae* thrived in any media with or without mucin, indicating that host-derived substrates alone are insufficient to maintain mucin-degrading specialists.

Bile salts are common in the small intestine and play an important role in intestinal homeostasis by controlling the size and composition of the intestinal microbiota with its antimicrobial effect. A wide variety of bacteria express bile salt hydrolases that will modify the primary bile acids and thereby have a higher survival rate in the presence of bile acids (207). The addition of bile salts did increase the growth of *Enterobacteriaceae* and reduced the *Bifidobacteriaceae*, which was unexpected as genes for bile salt hydrolases have been found in Bacteroidota, Actinomycetota and Bacillota, and not much in Pseudomonadota (207). These somewhat non-cohesive results indicate the need of metagenomic analysis of the original fecal composition to ensure the best growth conditions for its metabolic

functional profile.

Because the simple medium produced the closest resemblance to the original fecal inoculum in multivariate space, it was selected for the bioreactor runs.

Across both experiments, reactors inoculated with different preparations (fecal slurry, supernatant, glycerol stocks) converged toward similar community states, with Chao1 richness stabilizing around 110-120 ASVs. This convergence suggests that the bioreactor imposes strong selective pressures that shape community assembly, while the initial inoculum still influenced long-term dynamics and specific species presence.

A shift from CO₂ to N₂ flushing induced a reproducible restructuring of the community (**Paper III, Fig. 4A,B**), although no single taxon explained the transition. Network analyses revealed that synergistic interactions among taxa, rather than individual species, drove the observed changes, with different hub taxa emerging in each experiment. This underscores the sensitivity of microbial communities to subtle environmental parameters such as redox conditions and gas composition.

Metronidazole treatment produced predictable declines in obligate anaerobes, including *Desulfovibrionaceae* and *Ruminococcaceae*, and triggered transient blooms of Enterobacteriaceae, although we did not observe an increase in *Bifidobacterium* which previously has been observed in metronidazole treatment (208,209). Although the magnitude of disruption was modest compared to *in vivo* settings, the reactors show the capacity to capture key ecological signatures of antibiotic perturbation. Differences between experiments indicated that initial community structure modulates antibiotic sensitivity and recovery trajectories.

Overall, the study establishes the bioreactor system as a robust and tractable platform for studying gut microbial ecology, antibiotic responses, and colonization resistance. It reliably maintains dominant intestinal families, supports controlled environmental manipulation, and enables reproducible perturbation experiments. The findings also highlight the importance of media composition, gas conditions, and inoculum characteristics in shaping community behavior. Incorporating metabolomics and metagenomics in future work would further strengthen the system's utility for mechanistic studies of microbial function and adaptation.

5 Concluding remarks and future perspectives

The papers in this thesis provide a small glimpse in the large field of microbiota studies, all the way from the clinic to *in vivo* to *in vitro*. We studied the early-life microbiota in correlation with asthma development and host influences in the mouse as well as how the microbiota can be studied in the lab. Together these papers add to the knowledge of the dynamics of the microbiota in the lab and in relation to the host.

In Paper I and II, we observed patterns in the microbial composition and potential functional capacity that could be connected to the hosts physiological status. In paper I we identified microbial patterns that correlated with immune patterns and later development of asthma in young adults. Similarly in Paper II we connected microbial community dynamics with host metabolic phenotypes, and we could identify consistent associations between the microbiota, microbial and host metabolites, and immune modulation *in vitro* as well as in the mice (153). Both studies gave a longitudinal picture of the microbiota, and an end point immune phenotype, with their direct connection being a “black box”. These results strengthen the need to continue study the mechanistic background of these interactions, especially during the window of opportunity.

The two-generation HMA model in Paper II provided us with such a possibility. Because it captures vertical transmission and early-life co-development of microbiota and host, it can be used to test targeted microbial interventions, both during pregnancy as well as during early-life. Using defined consortia (154), strains with traceable antibiotic resistance plasmids (210), or giving metabolite supplementation or antibiotics within this model at different time-points will give us insights in how specific microbial functions shape metabolic and immunological outcomes. Additionally, using genetically modified mice, such as humanized mice with more human like immune systems (211) will improve the results and make them more easily translatable to the clinic.

Paper III demonstrates that anaerobic bioreactor systems can maintain complex microbial communities and reproduce ecological dynamics under controlled conditions. These systems offer a unique opportunity to study microbial interactions, colonization resistance, and antibiotic responses without host confounders. Future research should include functional readouts, such as metabolite fluxes, SCFA production, and resistance gene expression and spread, to link community shifts with ecological and metabolic consequences. Additionally, the environment of the bioreactor could be improved to mimic the GI tract even better, potentially by adding a solid phase for bacteria to bind to. Deeper analysis of the community could also be done by metagenomics to track adaptive responses, niche specialization, and resistance evolution in real time. Unfortunately, these analyses were not possible during my PhD studies.

Ultimately, taking observations from the clinic, and then coupling bioreactors with HMA models could create a powerful pipeline for testing hypotheses generated *in vitro* and validating them *in vivo* to confirm the observations from the clinic.

Across all three papers, a recurring theme is the importance of ecological context.

Microbial communities do not act in isolation; their effects on the host depend on community structure, metabolic networks, and environmental conditions. Future research should therefore embrace ecological and systems-biology frameworks, focusing on microbial interactions, cross-feeding networks, and the resilience of microbial ecosystems under perturbation. This perspective will be essential for designing effective microbiota-based therapies, whether aimed at preventing allergic disease, restoring microbial balance after antibiotics, or modulating host metabolism.

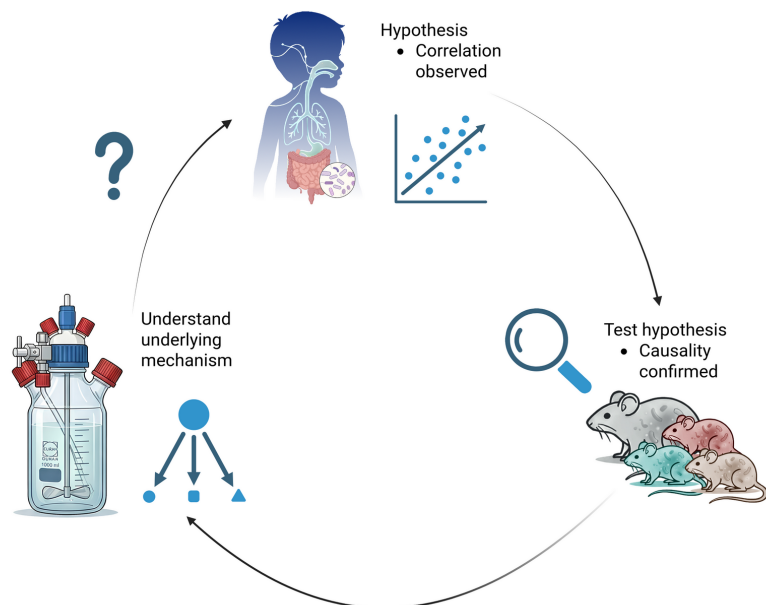


Figure 6: The circle of my research. From observing correlations in the clinic, creating a hypothesis, testing this in a two generational HMA mouse model and finally creating a tool to study the underlying mechanisms. *Created in Biorender.*

Finally, the translation of the results into the clinic is crucial. Identifying early-life microbial or metabolic signatures that predict allergy risk could inform preventive strategies, such as maternal dietary interventions, targeted probiotics, or early-life microbial supplementation. Understanding how antibiotics reshape microbial ecosystems may guide more precise clinical use and support the development of microbiota-sparing therapeutics.

In summary, the findings of this thesis underscore the continuous need for interdisciplinary approaches that combine human cohort studies, mechanistic animal models, and controlled *in vitro* systems. By bridging these levels of investigation, future research can move closer to unraveling the causal pathways through which the early-life microbiota shapes lifelong health and ultimately translate this knowledge into interventions that support resilient microbial ecosystems and balanced immune development.

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My path wasn't a straight line, but every detour led me here.

7 Declaration about the use of AI tools

I hereby declare that I used the AI tool Gemini (2.5 Flash) and Microsoft Copilot to support in the writing of this thesis, exclusively for the purpose of checking grammar and improve word choice. Literature research, conceptional design, data interpretation, and the writing itself were performed by myself, with support from my supervisor and co-authors of the included papers and manuscript.

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