Holistic Review of the Association Between Microbiome and Immune-Mediated Inflammatory Diseases

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Statement of Original Authorship

The work contained in this research has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made. I hereby declare that all the information in this research was obtained and presented in accordance with academic rules and ethical conduct. All the materials, various schools of thoughts and other academics consulted and are so quoted in these research work and dissertation are fully and duly acknowledged.

Signature: 

Date: _____28/02/2025 _____

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ABSTRACT

Background/Rationale:

The etiologic and pathological mechanisms of the immune mediated inflammatory disease (IMID) are still limited but there is a strong hypothesis about these diseases where they emerged as a reaction to environmental and genetic factors.[1]

Previously the association between the skin and gut microbiota has been investigated each time separately for some of the specific immune mediated disease (e.g. the association for gut microbiota and psoriasis (PsO), psoriatic arthritis (PsA) and the skin microbiota for inflammatory bowel disease (IBD))[2,3]. Since the data is limited, to date there aren't any systematic review to be able to understand holistically the relationship between different microbiota such as gut-skin and immune mediated diseases.

Aim:

The main objective of this research will be to review systematically the relationship between microbiome and immune mediated inflammatory diseases (IMIDs) such as rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, hidradenitis suppurativa, atopic dermatitis, inflammatory bowel disease (Crohn and ulcerative colitis). Secondly the research will focus to discuss the microbiome's role in the prevention, diagnosis, predictive and clinical value in disease monitoring among IMIDs.

Conclusion:

As this dissertation encapsulates the comprehensive insights derived from a systematic literature review that meticulously examined the intricate relationship between the microbiome

and immune-mediated inflammatory diseases (IMIDs) such as rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (IBD). Through a thorough synthesis of existing scientific evidence, this review elucidates the multifaceted interplay between microbial communities and the host immune system, underscoring the significance of the microbiome in the aetiology and progression of these complex and interconnected diseases. The identification of commonalities in the microbiome signatures associated with these IMIDs not only consolidates the existing knowledge base but also presents an opportunity for the development of unified therapeutic strategies targeting the shared aspects of pathophysiology. Moreover, this systematic review provides a holistic overview of the methodological approaches employed in microbiome research within the context of IMIDs, shedding light on gaps in current knowledge and suggesting directions for future investigations. As we navigate the complexities of the microbiome's impact on immune-mediated inflammation, this review not only informs our understanding of the existing literature but also serves as a roadmap for researchers, guiding them towards fruitful avenues for further exploration.

Chapter 1: Introduction and Aim of Study

In recent years, the intricate relationship between the human microbiome and immune-mediated inflammatory diseases (IMIDs) has emerged as a focal point in biomedical research, revolutionizing our understanding of the intricate crosstalk between microbial communities and host immune responses. Among the diverse spectrum of IMIDs, joint related conditions such as rheumatoid arthritis (RA), psoriatic arthritis; dermatological conditions such as psoriasis or atopic dermatitis and inflammatory bowel disease (IBD) stand out as complex and debilitating conditions, impacting millions worldwide.[1,2] This systematic research endeavors to delve deep into the molecular landscape, aiming to elucidate the pivotal role played by the microbiome in the pathogenesis and progression and potential management of these diseases.

The human microbiome, a diverse ecosystem of microorganisms inhabiting various niches within and on the human body, has been recognized as a dynamic player in maintaining homeostasis and influencing a multitude of physiological processes. Recent advancements in high-throughput sequencing technologies have facilitated comprehensive investigations into the composition and function of these microbial communities, uncovering their profound impact on immune system regulation. This research seeks to bridge the existing knowledge gap by exploring the intricate interplay between the microbiome and the development, exacerbation, or attenuation of above mentioned IMIDs.[1,2]

Despite their distinct clinical manifestations, emerging evidence suggests commonalities in the dysregulation of immune responses and microbial imbalances across these IMIDs.[1-3] By scrutinizing the molecular mechanisms underlying the microbiome-immune system axis in

these diseases, this research aims to uncover potential therapeutic targets and innovative strategies for disease management.

As we embark on this academic journey, the integration of cutting-edge technologies and interdisciplinary approaches will be pivotal in unraveling the complexities of the microbiome's impact on the immune-mediated inflammatory landscape. Through this systematic review, it's aimed to aspire to contribute valuable insights that not only deepen our understanding of the pathophysiology of IMIDs but also pave the way for the development of personalized and targeted interventions, ushering in a new era of precision medicine in the realm of autoimmune and inflammatory diseases.

1.1 BACKGROUND

The etiologic and pathological mechanisms of the immune mediated inflammatory disease (IMID) are still limited but there is a strong hypothesis about these diseases where they emerged as a reaction to environmental and genetic factors.[1]

Previously the association between the skin and gut microbiota has been investigated each time separately for some of the specific immune mediated disease (e.g. the association for gut microbiota and psoriasis, psoriatic arthritis and the skin microbiota for inflammatory bowel disease)[2,3]. Since the data is limited, to date there aren't any systematic review to be able to understand holistically the relationship between different microbiota such as gut-skin and immune mediated diseases.

1.2 STUDY OBJECTIVES

Main objective of this research is to review systematically the relationship between microbiome and immune mediated inflammatory diseases (IMIDs) such as rheumatoid arthritis,

psoriasis, psoriatic arthritis, hidradenitis suppurativa, atopic dermatitis, inflammatory bowel disease (Crohn and ulcerative colitis). Secondary objective of this research is to discuss the microbiome's role in the prevention, diagnosis, predictive and clinical value in disease monitoring among IMIDs.

1.3 SIGNIFICANCE, SCOPE AND DEFINITIONS

To date there has been some research that have been conducted either individually in order to search the link between the microbiome and separate immune mediated diseases such as only rheumatoid arthritis or only psoriasis. This study as claimed; aimed to search the association of microbiome between the immune mediated diseases by including more immune mediated diseases. In this study the most common dermatological, articular and gastroenterological inflammatory chronical immune-mediated diseases have been chosen in order to associate this link. But neurological or hormonal chronical diseases are not in scope of this research. This research includes the following IMIDs; rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, hidradenitis suppurativa, atopic dermatitis, inflammatory bowel diseases (Crohn and ulcerative colitis).

Chapter 2: Methodology

The current literature searching the link between the microbiome and immune mediated disease were the main source of this holistic review. Initially, a search of electronic database was conducted, and 2550 possible studies have been included coming from different databases such as PubMed, Google Scholar (Figure 2.1). 485 of these publications were matched the eligibility criteria based on the relevancy along with excluding other inflammatory chronic diseases such as uveitis or neurological chronic inflammatory diseases. As stated above these conditions are subjects to exclusion criteria for this research. Finally, an additional filtering has been applied based on the context and titles and 146 publications have met the eligibility criteria for this holistic review.

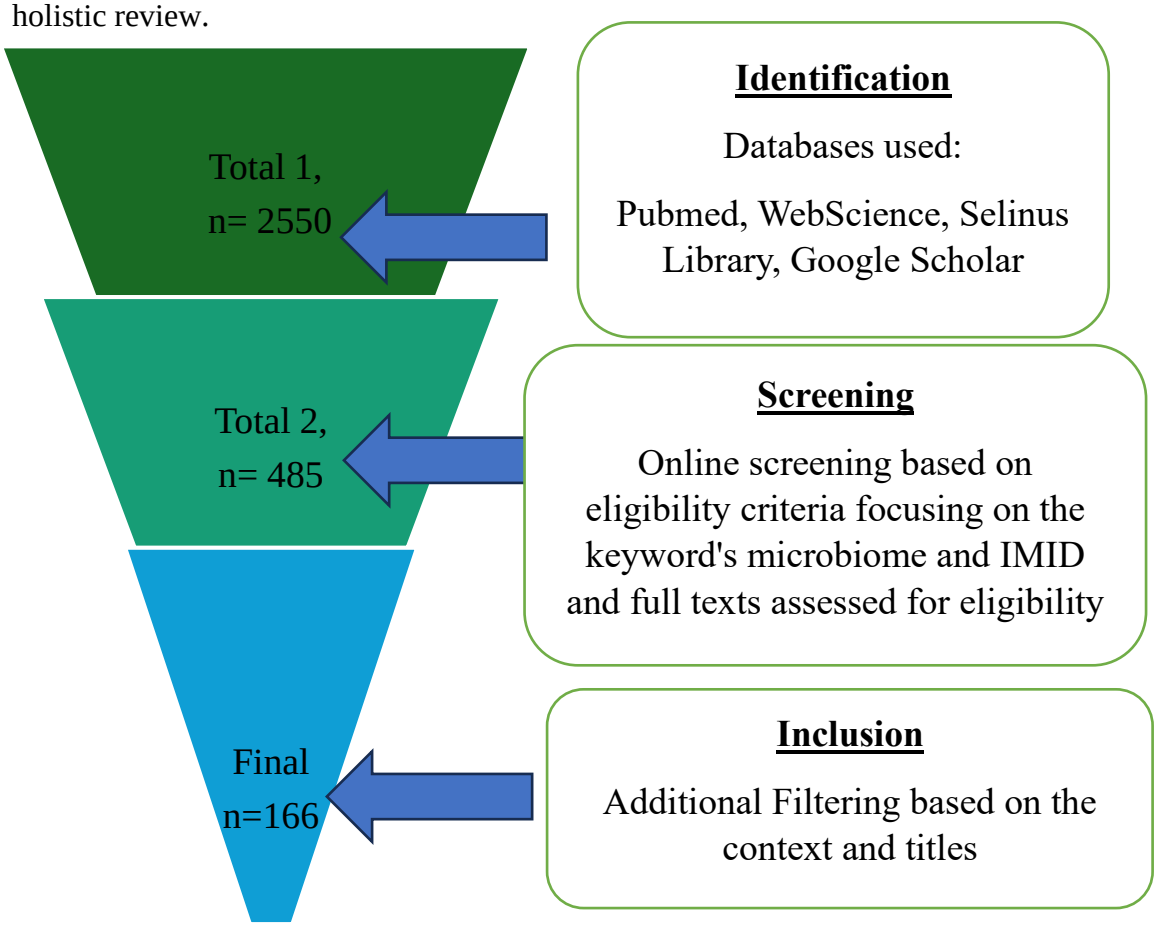

Figure 2.1. Schematization for the literature screening

Chapter 3: Literature Reviews

3.1 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder primarily targeting the synovial joints, resulting in inflammation, pain, and progressive joint damage. The etiopathogenesis of RA is multifaceted, involving a complex interplay between genetic, environmental, and immunological factors.[4]

Genetically, RA is closely linked with specific alleles of the human leukocyte antigen (HLA) system, particularly HLA-DRB1. These genetic markers are associated with both susceptibility to and severity of the disease, with the shared epitope hypothesis suggesting that certain sequences within these alleles predispose individuals to autoimmunity. Beyond the HLA region, genome-wide association studies (GWAS) have identified numerous other genetic loci that contribute to the polygenic nature of RA. [4,5]

Environmental factors are also critical in the onset of RA. Smoking is the most extensively documented environmental risk factor, significantly increasing the likelihood of developing RA, especially in genetically susceptible individuals. Other environmental triggers include microbial agents such as Epstein-Barr virus and periodontal pathogens like *Porphyromonas gingivalis*, which can induce protein citrullination, a key process in RA pathogenesis. Dysbiosis, or an imbalance in the oral, bronchial and gut microbiota, has been implicated in altering the immune system's behaviour, potentially triggering autoimmune responses in genetically predisposed individuals.[4]

Immunologically, RA is characterized by the presence of autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs), which often precede clinical symptoms by years (Figure 3.2). The autoimmune response in RA involves the activation of T-cells, B-cells, and macrophages, leading to the production of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6). These cytokines drive the inflammatory process, resulting in synovitis and the formation of pannus, an abnormal fibrovascular tissue layer that invades and destroys cartilage and bone.[4]

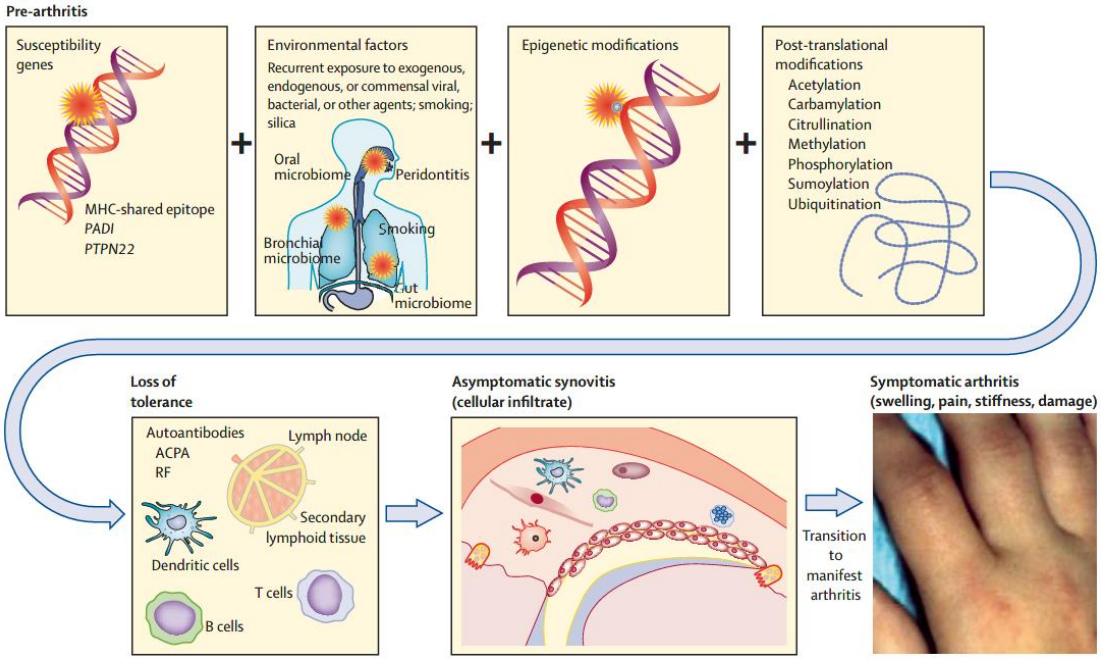

Figure 3.2. Etiopathogenesis for Rheumatoid Arthritis[4]

ACPA=autoantibodies against citrullinated peptides. RF=rheumatoid factor

The synovial environment in RA is marked by hyperplasia of synovial fibroblasts, increased vascularity, and infiltration by immune cells. This chronic inflammatory state not only damages joint structures but also leads to systemic complications, including cardiovascular, pulmonary, and skeletal issues.[4]

In summary, the etiopathogenesis of rheumatoid arthritis involves a dynamic and intricate interaction of genetic predisposition, environmental factors, and dysregulated immune

responses. Understanding these interconnected mechanisms is essential for developing targeted therapies and improving outcomes for patients with RA.

3.2 ANKYLOSING SPONDYLITIS

Ankylosing spondylitis (AS) is a debilitating inflammatory disease that primarily targets the spine and sacroiliac joints, leading to chronic pain and progressive stiffness. The pathogenesis of AS is intricately woven from genetic, environmental, microbial, and immunological threads, each contributing to the onset and progression of the disease.[6]

A significant genetic hallmark of AS is the strong association with the HLA-B27 allele. This genetic marker is found in the majority of individuals with AS, suggesting a pivotal role in disease susceptibility. HLA-B27 is hypothesized to influence immune system behaviour, potentially through antigen presentation or molecular mimicry. Beyond HLA-B27, advances in GWAS have identified additional genetic loci, such as ERAP1 and IL23R, that further elucidate the polygenic nature of AS and its varied clinical manifestations.[6,7]

Environmental influences also play a crucial role in the development of AS. Infections, particularly those affecting the gastrointestinal and genitourinary tracts, are suspected to trigger or exacerbate the condition. The theory of molecular mimicry suggests that these infections may lead to an immune response that mistakenly targets the body's own tissues, perpetuating chronic inflammation characteristic of AS.[6]

Recent research has highlighted the gut microbiome as a significant factor in AS pathogenesis. Dysbiosis, or an imbalance in the gut microbial community, has been associated with immune dysregulation and increased inflammation. Specific gut bacteria may provoke a pro-inflammatory environment, thereby contributing to the autoimmune processes observed in AS.[6]

Immunologically, AS is marked by persistent inflammation at entheses, the sites where tendons and ligaments attach to bones. This enthesitis is driven by both innate and adaptive immune responses, with key pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-17 (IL-17) playing central roles (Figure 3.3). These cytokines stimulate osteoclast and osteoblast activity, leading to the dual pathological processes of bone erosion and abnormal new bone formation that define AS.[6]

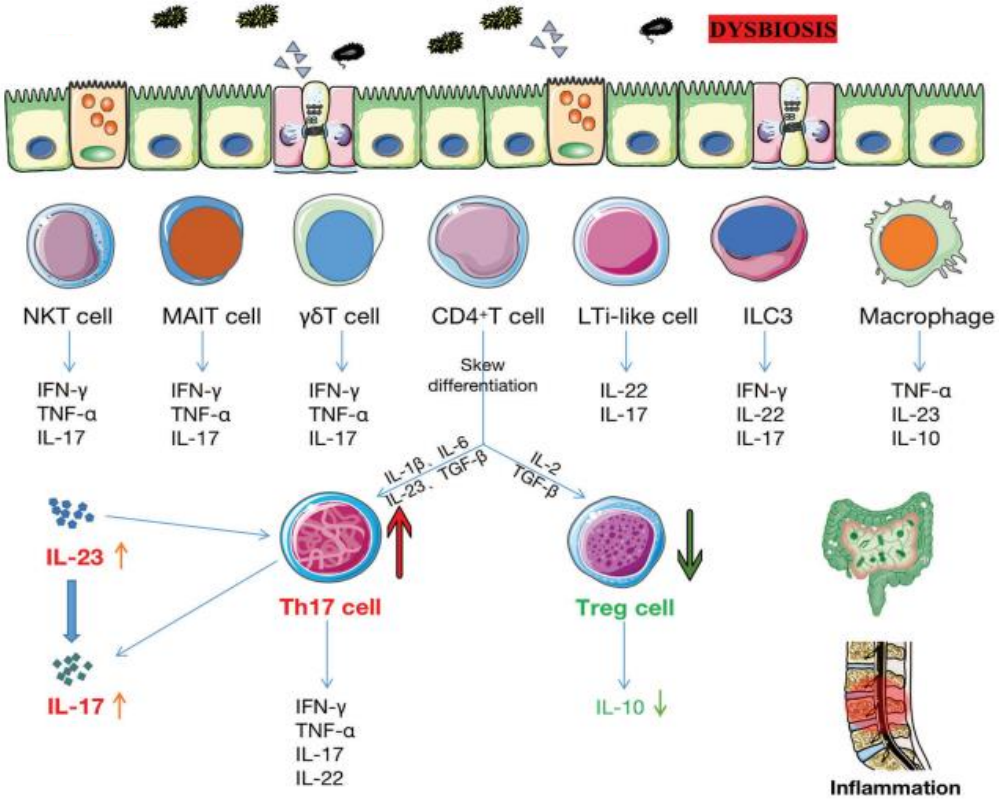

Figure 3.3 Microbiome dysbiosis triggers immune cells to increase proinflammatory cytokines in Ankylosing Spondylitis[8]

Th17: T helper 17 cells; Treg: T regulatory cell; ILC3: innate lymphoid cell 3; MAIT: mucosal-associated invariant T cell; NKT: natural killer T cell; IL-17: interleukin 17; TNF-a: tumor necrosis factor-a; IFN-g: interferon-g.

In summary, the etiopathogenesis of ankylosing spondylitis is a complex interplay of genetic predisposition, environmental triggers, microbial factors, and immune system dysregulation. A comprehensive understanding of these interconnected mechanisms is vital for developing effective therapeutic strategies and improving patient outcomes.

3.3 PSORIATIC ARTHRITIS

Psoriatic arthritis (PsA) is a multifaceted chronic inflammatory disorder characterized by the involvement of both musculoskeletal and cutaneous systems and commonly accompanies psoriasis. The pathogenesis of PsA is deeply intertwined with genetic predispositions, environmental exposures, immune system aberrations, and microbial dysbiosis. A thorough understanding of these interlinked mechanisms is essential for advancing therapeutic approaches.[9]

PsA shares several genetic risk factors with psoriasis, particularly the HLA-Cw6 allele, which is strongly associated with both conditions. Beyond HLA-Cw6, genome-wide association studies have identified additional loci such as IL12B, IL23R, and TNIP1 that confer susceptibility to PsA. These genes are integral to the regulation of the IL-23/IL-17 axis, a critical pathway in PsA pathogenesis, influencing the production of pro-inflammatory cytokines that drive joint and skin inflammation (Figure 3.4).[9,10]

Environmental factors are pivotal in triggering PsA in genetically predisposed individuals. Trauma to the skin, known as the Koebner phenomenon, can precipitate PsA by initiating local inflammatory responses. Infections, particularly streptococcal pharyngitis, have been implicated in exacerbating PsA, possibly by molecular mimicry or by directly activating immune pathways.[9]

Emerging evidence suggests that the gut microbiome plays a crucial role in the pathogenesis of PsA. Dysbiosis, characterized by a decrease in anti-inflammatory bacterial taxa such as Bacteroides and an increase in pro-inflammatory species, has been observed in PsA patients. This microbial imbalance may contribute to systemic inflammation through mechanisms involving the gut-joint axis, where microbial metabolites and translocated bacterial products can modulate immune responses in distant sites, including joints. The exact nature of this gut-joint communication remains an active area of research, with studies suggesting that microbial antigens may activate dendritic cells and promote Th17 cell differentiation, leading to sustained inflammation.[9]

The immune response in PsA is marked by the activation of Th1 and Th17 cells, which are central to the disease's pathophysiology. The IL-23/IL-17 axis is particularly critical, with IL-17A driving neutrophil recruitment and activation in the synovium, leading to joint inflammation. TNF-α also plays a significant role by promoting the production of other pro-inflammatory cytokines and sustaining the chronic inflammatory environment. The role of IL-22, another cytokine associated with the Th17 response, has also been implicated in the keratinocyte hyperproliferation seen in psoriatic skin lesions (Figure 3.5). [9,11]

In PsA, the confluence of genetic predispositions, environmental insults, microbial dysbiosis, and immune dysregulation culminates in a chronic inflammatory state affecting both joints and skin. Understanding these complex interactions is essential for developing targeted therapies that can address the multifactorial nature of PsA.

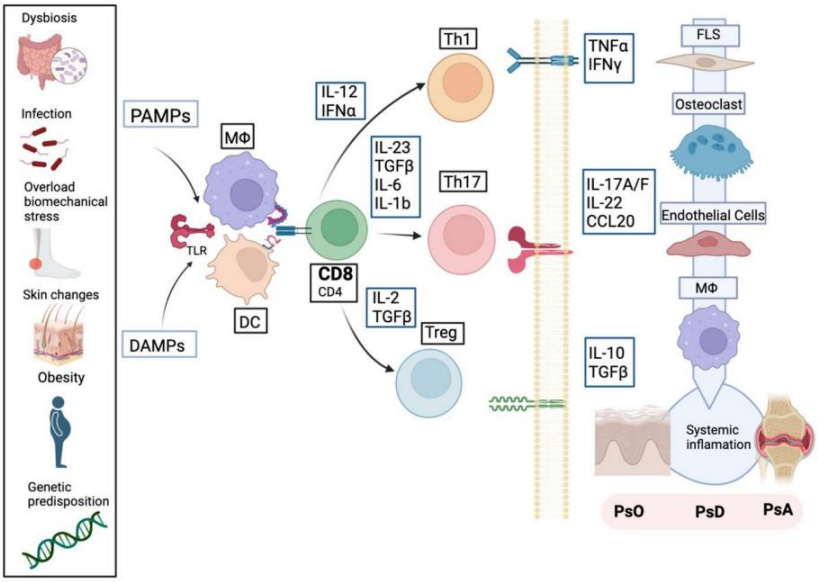

Figure 3.4. Pathophysiological Mechanisms in Psoriatic Diseases (PSD) [9] DAMPS (Damage-associated molecular pattern), PAMPs (Pathogen-associated molecular patterns), DC (dendritic cells), MΦ (Macrophages), CD8 (CD8 T lymphocyte), CD4 (CD4 T lymphocyte), Th1 (T helper 1 cells), Th17 (T helper 17 cells), Treg (T regulatory), FLS (synovial fibroblast).

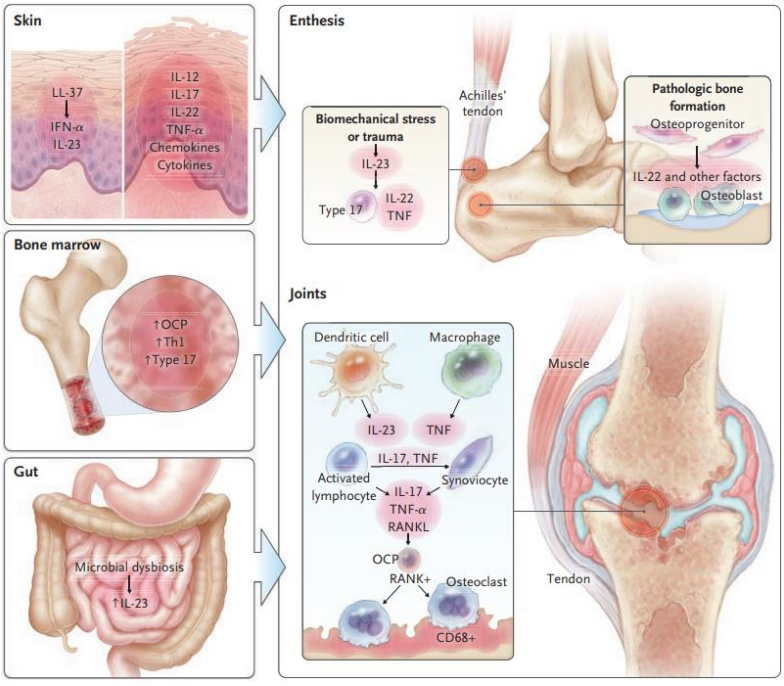

Figure 3.5. Pathophysiological Mechanisms in Psoriatic Arthritis (PsA) [11]

NF-κB (RANK) ligand (RANKL), osteoclast precursors (OCPs).

3.4 PSORIASIS

Psoriasis is a chronic, immune-mediated disease primarily affecting the skin, characterized by hyperproliferation of keratinocytes and sustained inflammation. The etiopathogenesis of psoriasis involves a multifactorial interplay between genetic predispositions, environmental factors, microbial influences, and immune system dysregulation.[12]

Psoriasis has a robust genetic component, with more than 60 susceptibility loci identified, particularly through GWAS. The HLA-Cw6 allele located within the major histocompatibility complex (MHC) on chromosome 6, remains the most significant genetic marker, especially in early-onset psoriasis. This allele influences the presentation of antigens to T cells, a critical step in initiating the immune response seen in psoriasis. Other important immune-related genetic contributors include IL12B and IL23R, both of which are involved in the IL-23/IL-17 axis, a critical pathway in the inflammatory cascade of psoriasis. Variants in IL23R are particularly associated with reduced susceptibility, suggesting its protective role when functioning optimally. Additionally, genes such as TNFAIP3, which is involved in the NF-κB signaling pathway, further underscore the genetic complexity of this disease (Figure 3.6). The interplay between these genetic contributors illustrates the multifactorial nature of psoriasis, where immune dysregulation, keratinocyte dysfunction, and environmental triggers converge. These genetic insights are critical for developing targeted therapies and understanding individual variability in disease presentation and response to treatment. [10,12,13]

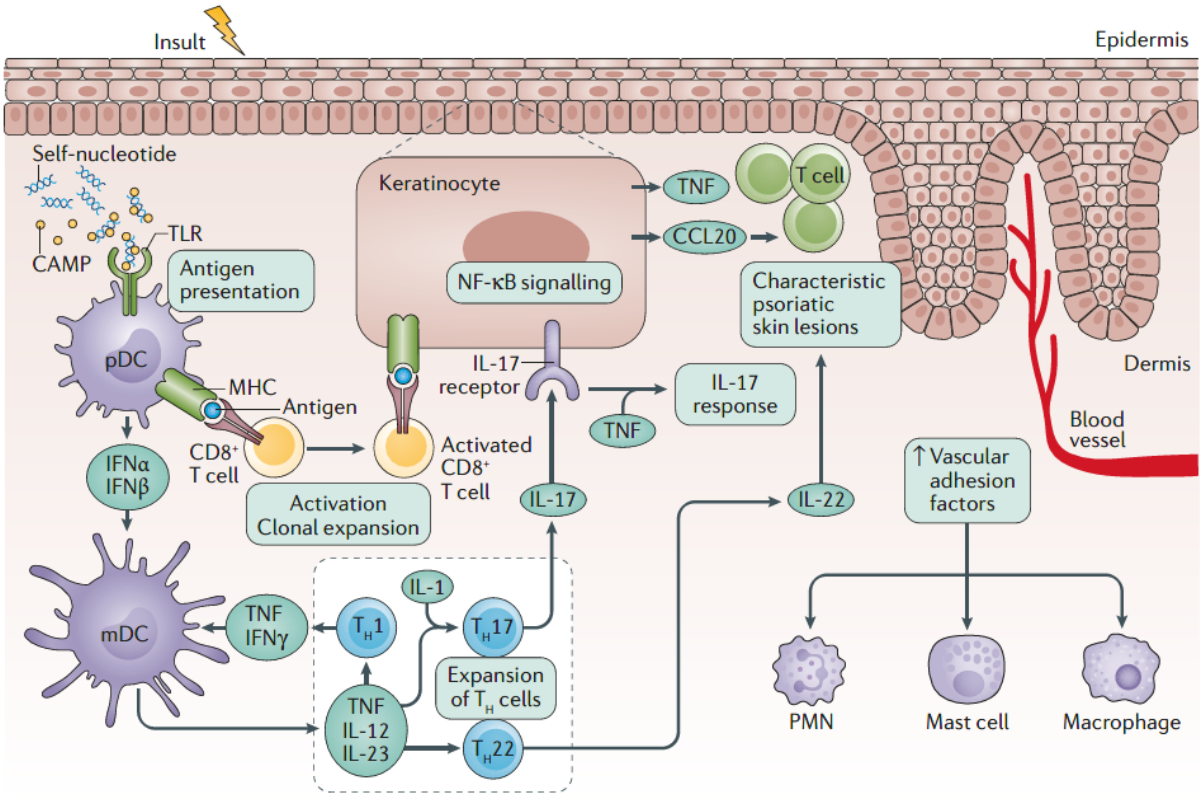

Figure 3.6. Pathophysiological Mechanisms in Psoriasis (PsO) [12]

Antimicrobial peptides (AMPs), cathelicidin antimicrobial peptide (CAMP), Toll-like receptor (TLR), plasmacytoid dendritic cells (pDCs), class I major histocompatibility complex (MHC), inflammatory mediators interferon-α (IFNα) and IFNβ, myeloid DCs (mDCs), tumour necrosis factor (TNF). CC-chemokine ligand 20 (CCL20), the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) and nuclear factor-κB (NF-κB).

Environmental factors such as infections (notably streptococcal infections), mechanical injury, stress, and lifestyle choices like smoking can precipitate or exacerbate psoriasis in genetically predisposed individuals. These triggers often initiate a pathological immune response, particularly in individuals carrying susceptible genetic markers. The Koebner phenomenon, where trauma to the skin leads to psoriatic lesions, exemplifies how environmental factors can activate disease mechanisms.[12]

The microbiome, especially the skin and gut microbiota, plays a pivotal role in modulating immune responses in psoriasis. Dysbiosis in the gut, characterized by a decrease in Firmicutes and an increase in Actinobacteria, has been linked to psoriasis, suggesting a systemic influence on skin inflammation. On the skin, the microbiome is often altered in psoriatic patients, with a relative increase in Streptococcus and Propionibacterium species. These microbial changes can influence local immune responses, potentially through the activation of Toll-like receptors (TLRs) on keratinocytes and dendritic cells, leading to the production of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. [12,14]

Psoriasis is characterized by a dysregulated immune response, particularly involving the Th1 and Th17 pathways. The IL-23/IL-17 axis is central to psoriasis pathogenesis, with IL-17A being a key cytokine driving keratinocyte proliferation and neutrophil infiltration into the dermis. Th1 cells contribute to the inflammatory milieu by producing IFN-γ, which further amplifies the immune response. Additionally, the role of Th22 cells and their production of IL-22 highlights the complexity of the immune network involved in psoriasis, contributing to epidermal hyperplasia and impaired barrier function. [12]

Psoriasis arises from a complex interaction of genetic predisposition, environmental triggers, microbial dysbiosis, and immune dysregulation. Understanding these factors is crucial for developing targeted therapies that address the underlying causes of the disease and improve patient outcomes.

3.5 HIDRADENITIS SUPPURATIVA

Hidradenitis suppurativa (HS) is a chronic, disabling skin disorder characterized by recurrent, painful nodules, abscesses, and sinus tracts, predominantly affecting apocrine gland-

rich areas. The pathogenesis of HS is multifactorial, involving genetic predispositions, environmental factors, microbial dysbiosis, and immune system dysregulation.[15]

Genetic factors play a significant role in HS, with familial clustering observed in a subset of patients. Mutations in genes encoding components of the gamma-secretase complex, such as NCSTN, PSEN1, and PSENEN, have been identified in some patients, highlighting the importance of the Notch signaling pathway in skin homeostasis and immune function. These mutations are associated with aberrant keratinocyte differentiation and hyperkeratosis, leading to follicular occlusion and the formation of primary HS lesions.[15,16]

Environmental factors such as obesity, smoking, and mechanical friction are strongly associated with HS and can exacerbate disease severity. These factors contribute to the disruption of follicular units, promoting inflammation and secondary bacterial colonization. Smoking, in particular, has been linked to increased oxidative stress and altered immune responses, which can further aggravate HS.[15]

The skin microbiome is significantly altered in HS, with dysbiosis characterized by an overrepresentation of Staphylococcus aureus, Streptococcus species, and anaerobes such as Propionibacterium acnes. These microbial changes may contribute to the chronic inflammation observed in HS by interacting with the innate immune system. Toll-like receptors (TLRs) on keratinocytes and immune cells recognize microbial components, leading to the activation of NF-κB and the subsequent production of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-17 (Figure 3.7- Figure 3.8). The chronicity of HS may be further sustained by biofilm formation, which can protect bacteria from immune clearance and antibiotic treatment.[15]

HS is characterized by a dysregulated immune response, particularly involving the innate immune system. Neutrophil infiltration and the release of neutrophil extracellular traps

(NETs) contribute to tissue damage and perpetuate the inflammatory cycle. The role of Th17 cells and the associated production of IL-17 is increasingly recognized as a key factor in HS pathogenesis, driving both the local inflammatory response and systemic immune activation. Additionally, elevated levels of TNF-α have been found in HS lesions, supporting the use of TNF inhibitors in the treatment of severe cases.[15]

The pathogenesis of hidradenitis suppurativa is driven by a complex interplay of genetic mutations, environmental factors, microbial dysbiosis, and immune dysregulation. Understanding these mechanisms provides a foundation for developing more effective treatments that target the underlying causes of this debilitating condition.

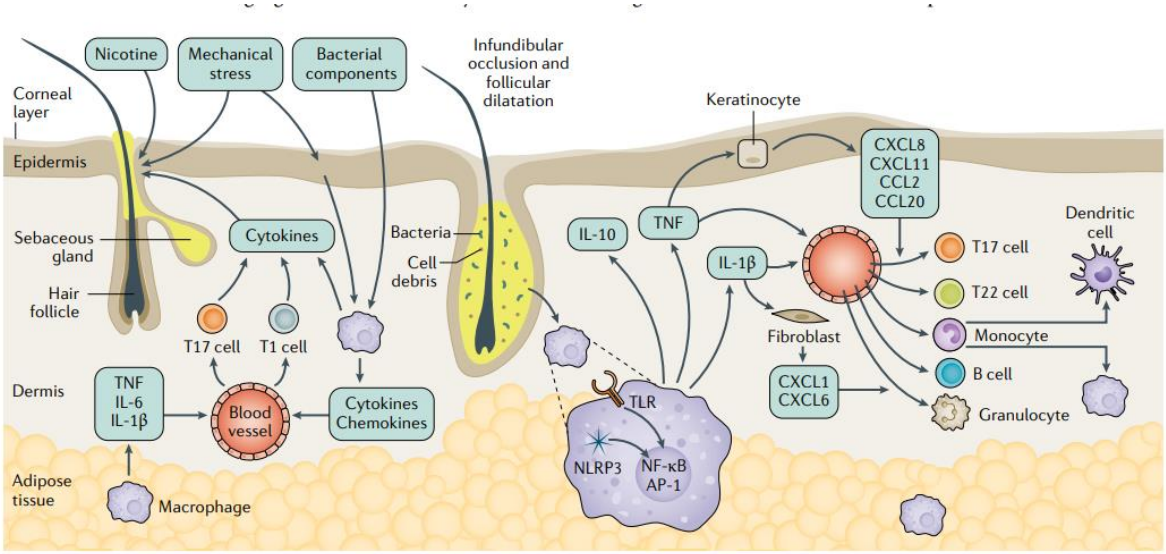

Figure 3.7. Initial Pathophysiological Mechanisms in Hidradenitis Suppurativa[15]

AP-1, activator protein 1 (heterodimeric transcription factor); NLRP3, NACHT, LRR and PYD domain-containing protein 3 (inflammasome component, also known as cryopyrin); NF-κB, nuclear factor-κB (transcription factor); TLR, Toll-like receptor.

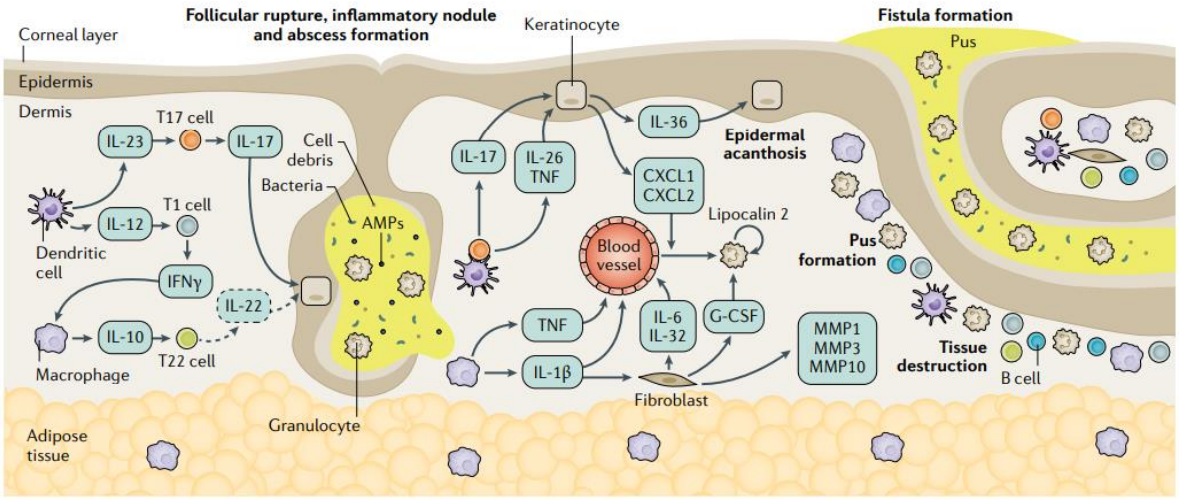

Figure 3.8. Progressed Pathophysiological Mechanisms in Hidradenitis Suppurativa[15]

Interleukin (IL), interferon-γ (IFNγ), T helper (TH) cells and cytotoxic T (TC) cells, antimicrobial protein (AMPs), matrix-degrading enzymes (matrix metalloproteinases (MMPs)), granulocyte colony-stimulating factor (G-CSF), tumour necrosis factor (TNF)

3.6 ATOPIC DERMATITIS

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder characterized by intense pruritus, eczematous lesions, and a significant impact on the quality of life. The etiopathogenesis of AD is multifactorial, involving genetic predispositions, environmental factors, microbiome alterations, and immune system abnormalities.[17]

Genetic predisposition plays a critical role in AD, particularly through mutations in the filaggrin (FLG) gene, which encodes a key protein involved in maintaining the skin barrier. Loss-of-function mutations in FLG result in a compromised skin barrier, increasing trans epidermal water loss and facilitating the penetration of allergens and microbes. This barrier dysfunction is a hallmark of AD and contributes to the chronic inflammation observed in patients. Additionally, other genetic loci associated with AD include those involved in immune

regulation, such as IL-4 and IL-13, which are central to the Th2-skewed immune response in AD. [17]

Environmental factors such as exposure to allergens (e.g., dust mites, pollen), irritants (e.g., soaps, detergents), and climatic conditions can exacerbate AD by further compromising the skin barrier and triggering immune responses. The “hygiene hypothesis” suggests that reduced microbial exposure early in life may predispose individuals to atopic conditions, including AD, by skewing the immune system toward a Th2-dominant response. [17]

The skin microbiome in AD patients is markedly different from that of healthy individuals, with a significant reduction in microbial diversity and a predominance of Staphylococcus aureus. This dysbiosis may exacerbate the inflammatory response by stimulating the production of pro-inflammatory cytokines and antimicrobial peptides, which in turn can lead to further barrier disruption and perpetuate the cycle of inflammation. The role of S. aureus in AD is particularly significant, as its colonization correlates with disease severity and flare-ups. [17]

AD is characterized by a skewed immune response, predominantly driven by Th2 cells in the acute phase and a mixed Th2/Th22 response in the chronic phase. IL-4 and IL-13 are central cytokines in the Th2 response, promoting IgE production, eosinophil activation, and barrier dysfunction (Figure 3.9). IL-22, associated with Th22 cells, contributes to epidermal hyperplasia and reduced expression of filaggrin, exacerbating the barrier defect. In addition to Th2 and Th22 cells, the role of Th17 and Th1 cells becomes more prominent in chronic lesions, indicating a complex and evolving immune landscape in AD. [17]

Atopic dermatitis is a complex disorder arising from a combination of genetic predispositions, environmental factors, microbiome alterations, and immune dysregulation. Advances in

understanding these mechanisms have led to the development of targeted therapies that address the underlying causes of AD, offering hope for improved management of this chronic condition.

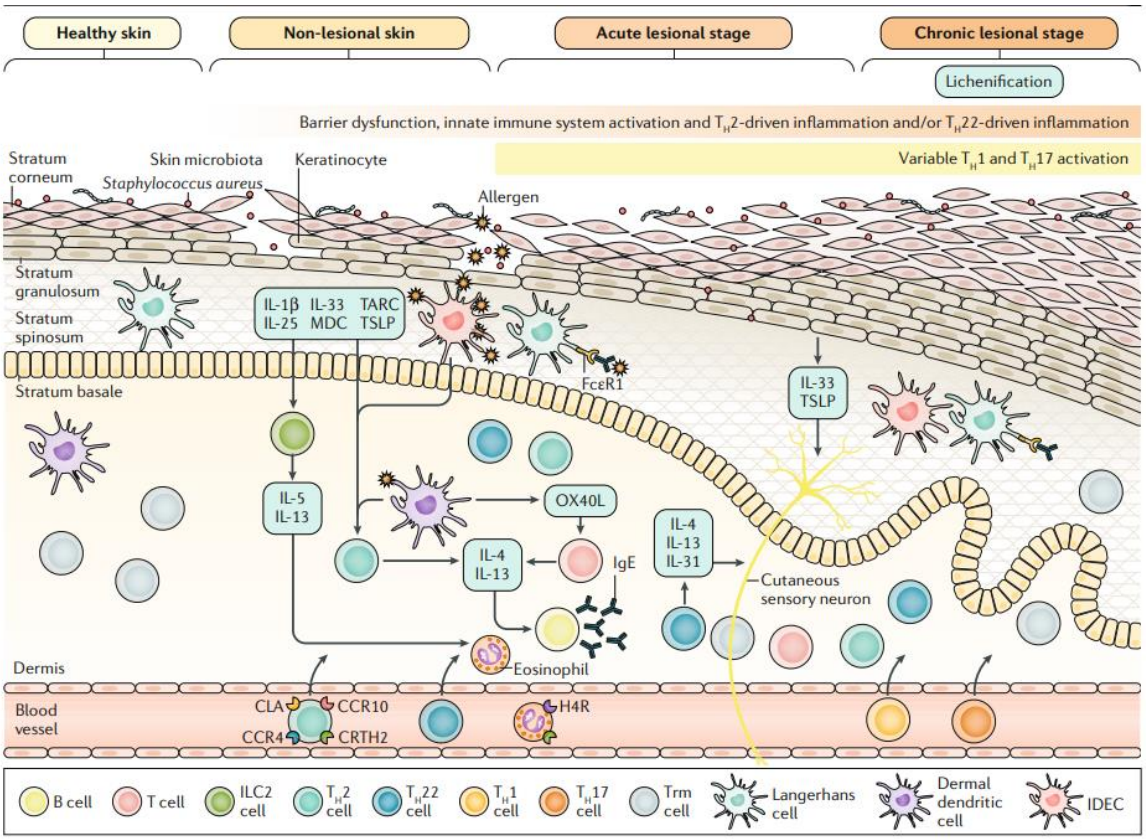

Figure 3.9. Pathophysiological Mechanisms in Atopic Dermatitis [17]

Thymus and activation-regulated chemokine (TARC; also known as CC-chemokine ligand 17 (CCL17)) and macrophage-derived chemokine (MDC; also known as CCL22) , as well as innate immune cytokines such as IL-1β, IL-33 and thymic stromal lymphopoietin (TSLP), skin-resident group 2 innate lymphoid cells (ILC2s) and T helper 2 cell (TH2), expression by dendritic cells of OX40 ligand (OX40L; also known as tumour necrosis factor ligand superfamily member 4 (TNFSF4)), inflammatory dendritic epidermal cells (IDECs), immunoglobulin E (IgE), cutaneous lymphocyte antigen (CLA), chemokine (CC-chemokine receptor 4 (CCR4) and CCR10) and lipid (chemoattractant receptor-homologous molecule expressed on TH2 cells (CRTH2; also known as prostaglandin D2 receptor 2)) chemoattractant receptors. Eosinophils expressing CRTH2 and histamine H4 receptor (H4R), high-affinity immunoglobulin-ε receptors (FcεR1s).

3.7 INFLAMMATORY BOWEL DISEASES

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), are chronic, multi-faceted inflammatory disorders of the gastrointestinal (GI) tract. The etiopathogenesis of IBD involves a multifactorial interplay of genetic predispositions, environmental influences, microbial dysbiosis, and immune system dysregulation.[18]

Genetic predisposition is a significant factor in IBD, with over 200 susceptibility loci identified through GWAS. Key genes implicated include NOD2, IL23R, and ATG16L1. NOD2, associated predominantly with Crohn's disease, plays a critical role in recognizing bacterial peptidoglycan and activating the immune response. Mutations in NOD2 lead to impaired microbial recognition and an exaggerated immune response. IL23R, involved in the Th17 pathway, influences the production of IL-17 and IL-22, cytokines central to the inflammatory process in IBD. ATG16L1, involved in autophagy, is essential for the clearance of intracellular pathogens, and its dysfunction can lead to abnormal immune responses to gut microbes. [18]

Environmental factors, including diet, smoking, antibiotic use, and infections, significantly impact the development and course of IBD. Smoking is particularly associated with Crohn's disease, while it tends to have a protective effect against ulcerative colitis. These factors can modulate the gut environment and interact with genetic predispositions to trigger or exacerbate inflammation. The Western diet, high in fats and refined sugars, has also been implicated in altering gut microbiota and promoting dysbiosis, thereby contributing to the pathogenesis of IBD. [18]

The gut microbiome plays a central role in IBD pathogenesis. Dysbiosis, characterized by a reduction in beneficial bacteria such as Firmicutes and Bacteroidetes and an increase in

pathogenic bacteria like Escherichia coli, disrupts the immune-microbiome balance. This microbial imbalance can lead to an inappropriate immune response, characterized by the activation of pro-inflammatory pathways, including the Th1 and Th17 pathways in Crohn's disease, and Th2 and Th17 in ulcerative colitis. Microbial translocation across the compromised intestinal barrier can further exacerbate inflammation by triggering innate immune responses. [18]

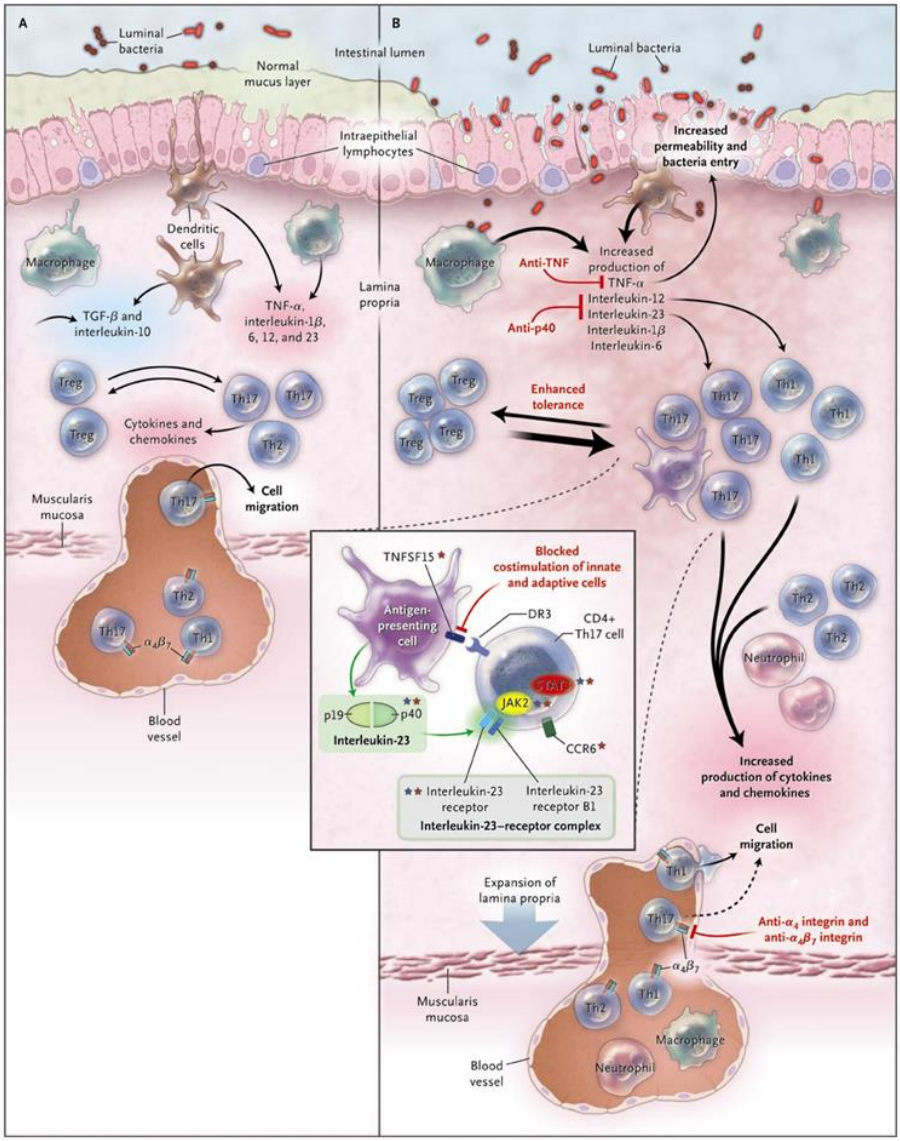

Figure 3.10. Pathophysiological Mechanisms in Inflammatory Bowel Disease [18]

The immune response in IBD is marked by chronic inflammation driven by an interplay between the innate and adaptive immune systems. In Crohn's disease, the Th1 and Th17 responses predominate, with increased production of TNF-α, IFN-γ, and IL-17, leading to granuloma formation and transmural inflammation. In ulcerative colitis, the immune response involves a mixed Th2 and Th17 profile (Figure 3.10), with elevated levels of IL-13 and IL-17 contributing to mucosal inflammation and ulceration. The dysregulated immune response in IBD leads to tissue damage, ulceration, and, in severe cases, fibrosis and strictures. [18]

Inflammatory bowel diseases are the result of a complex interaction of genetic susceptibility, environmental factors, microbial dysbiosis, and immune system dysregulation. Understanding these mechanisms is crucial for the development of targeted therapies that can effectively manage and treat IBD.

3.8 THE HUMAN MICROBIOME: DEFINITION, COMPOSITION AND DIVERSITY

The concept of the human microbiome, a term referring to the collective genomes of the microorganisms that reside within the human body, has revolutionized our understanding of human biology and health. These microorganisms, including bacteria, viruses, fungi, and archaea (Figure 3.11), inhabit various niches of the body, such as the skin, mouth, gut, and urogenital tract. Their contributions to human health are profound, influencing digestion, immune function, and even mental health.[19,20]

Historically, the human body was viewed as a largely sterile environment, with microorganisms considered merely as invaders that could cause disease. However, advances in molecular biology and genomic sequencing have unveiled the complexity and significance of the microbiome, marking a paradigm shift in medical and biological sciences. The development of next-generation sequencing technologies has been pivotal in this transformation, allowing

researchers to explore the microbial diversity within humans without the need for traditional culturing methods. [19-21]

This introduction to the microbiome sets the stage for an in-depth exploration of its role in health and disease (Figure 3.12). This thesis will examine the microbiome in healthy individuals, explore how dysbiosis can lead to various pathologies, and discuss the potential for microbiome-based therapies. [19-21]

The human microbiome is composed of a vast array of microorganisms, with bacteria being the most abundant. The gut microbiome alone is estimated to contain over 35,000 different bacterial species. Among the major phyla present, Firmicutes and Bacteroidetes dominate, with other significant phyla including Actinobacteria and Proteobacteria.[21]

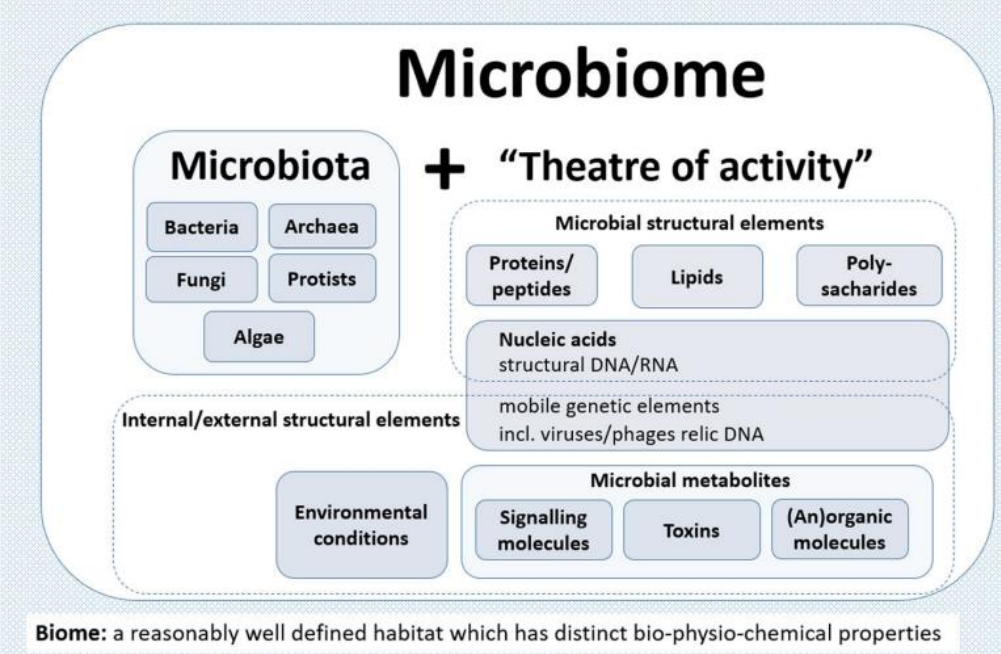

Figure 3.11 Schematization of Microbiome Composition [19]

The concept of the microbiome has evolved significantly over time, reflecting advancements in science and technology. Early microbiological studies in the 17th century, initiated by pioneers

like Antonie van Leeuwenhoek, revealed the presence of microorganisms in various environments, including the human body. However, these initial discoveries focused largely on pathogens rather than the broader microbial communities. The 20th century brought a shift in perspective, as researchers began to recognize the importance of commensal and symbiotic microbes in health and disease. The term “microbiome” itself was first introduced by Joshua Lederberg in 2001, emphasizing the collective genetic material of microorganisms within a particular environment and their role in human biology. Advances in molecular biology, particularly the advent of next-generation sequencing technologies, have since revolutionized microbiome research, allowing for detailed characterization of microbial communities across diverse body sites. This burgeoning field continues to uncover the profound impact of the microbiome on health, disease, and immune regulation, reshaping our understanding of human biology. [19-21] The composition of the microbiome is not static; it varies between individuals and can change over time. Factors such as genetics, diet, environment, age, and antibiotic use all influence the microbiome's composition. Despite this variability, healthy individuals typically share a core microbiome specific bacterial species that are consistently found across different people, suggesting that these core microbes play essential roles in maintaining health (Figure 3.13). The composition and abundance of the microbiome are dynamic and can vary substantially across different stages of life. [20]

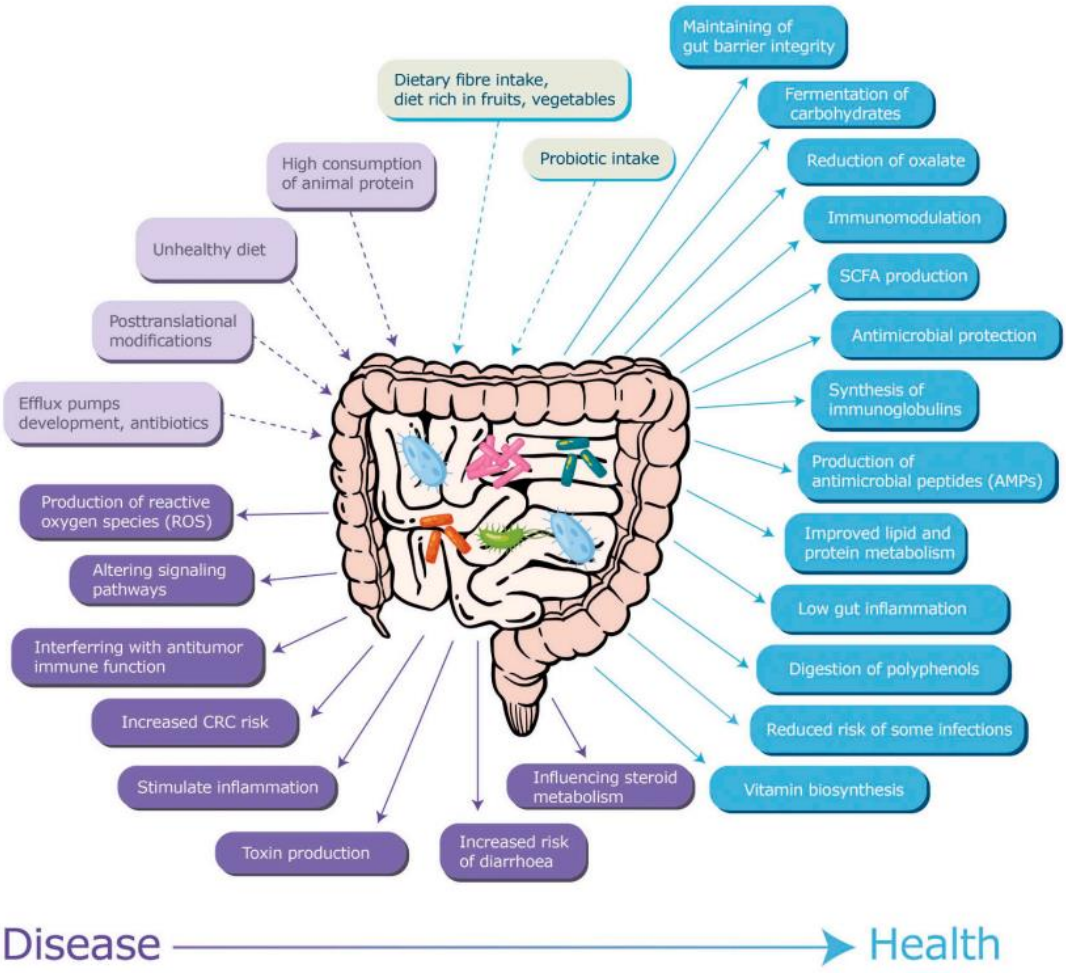

Figure 3.12. Overview of Microbiome Effects on Health and Disease [21]

CRC: Colorectal cancer, SCFA: Short chain fatty acid

During infancy, the microbiome is relatively undeveloped, with colonization beginning at birth and influenced by factors such as delivery mode, breastfeeding, and antibiotic use. In these early stages, microbial diversity tends to be low but increases as the infant's diet diversifies and the immune system matures. As individuals transition into adulthood, the microbiome reaches a more stable and diverse state, often reflecting long-term dietary patterns, lifestyle, and environmental exposures. Studies suggest that the adult microbiome is relatively stable,

although factors such as illness, diet changes, and antibiotic use can cause transient fluctuations (Figure 3.14). [22,23]

In contrast, the aging process is associated with a gradual decline in microbial diversity, often accompanied by shifts in the relative abundance of specific microbial taxa. These changes can be influenced by several factors unique to older adults, including decreased physical activity, altered dietary habits, increased medication use, and the weakening of immune function.

Research has shown that aging is often correlated with a reduction in beneficial microbes such as *Bifidobacterium* and *Lactobacillus*, and an increase in opportunistic pathogens. This shift in the microbiome composition has been hypothesized to contribute to age-related diseases and a general decline in health, including increased susceptibility to infections, chronic inflammation, and metabolic disorders. [22,23]

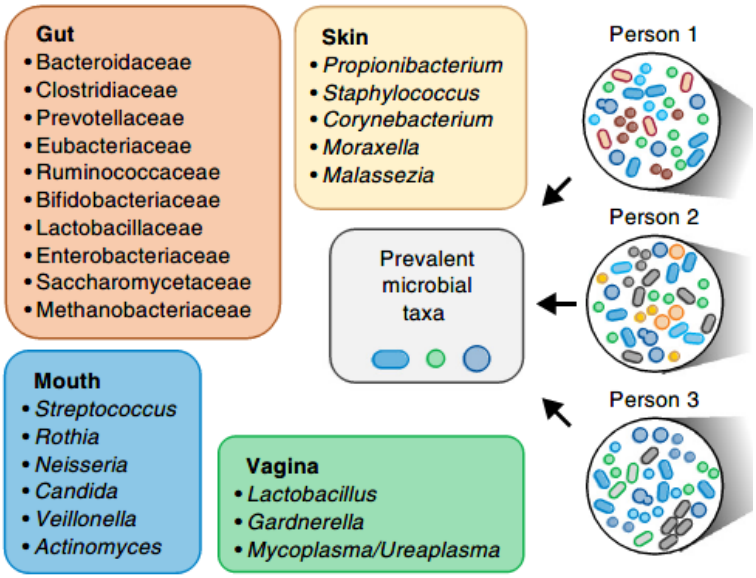

Figure 3.13. The taxonomy of the early definitions of healthy microbiome [20]

The colonization of the microbiome begins at birth, with factors such as delivery mode (vaginal birth vs. cesarean section) and feeding method (breastfeeding vs. formula feeding) playing

major roles in early microbial composition (Table 3.1). During infancy, the gut microbiome is dominated by Bifidobacterium, a genus of bacteria that metabolizes human milk oligosaccharides. This early colonization is essential for immune system development and gut health. [22,23]

However, microbial diversity remains low in the first few months of life, gradually increasing as the infant is exposed to new environmental stimuli and begins solid food consumption. Studies have shown that weaning leads to a marked increase in the abundance of Firmicutes and Bacteroidetes, two dominant phyla in the adult gut microbiome. This transition period sets the foundation for the more stable and diverse microbial communities seen in adulthood. [22,23]

As children grow, their microbiome continues to diversify and stabilize. By the time they reach adolescence, their microbiota resembles that of an adult. The microbiome of children is shaped by ongoing dietary and environmental exposures, including interactions with peers, school environments, and physical activity levels. [22,23]

During childhood, *Bacteroidetes* and *Firmicutes* become the dominant phyla, though their balance may fluctuate depending on diet and health status. For example, diets rich in fiber and plant-based foods tend to promote higher levels of *Bacteroidetes,* while high-fat, low-fiber diets are associated with an increased abundance of *Firmicutes*. These shifts in microbial populations during childhood and adolescence are crucial in shaping long-term health outcomes, as they influence metabolic processes and immune development. [22,23]

The adult microbiome tends to be more stable and diverse compared to earlier life stages, though it remains responsive to external factors such as diet, illness, and medication use. Firmicutes and Bacteroidetes are the predominant phyla in the adult gut, with Firmicutes often

being more abundant. This phylum includes a variety of genera such as *Clostridium*, *Lactobacillus*, and *Ruminococcus*, which are involved in the fermentation of dietary fibers, short-chain fatty acid production, and the regulation of gut health. [22,23]

In contrast, *Bacteroidetes*, which include genera like *Bacteroides* and *Prevotella*, play a key role in breaking down complex carbohydrates and maintaining energy balance. The relative abundance of these phyla has been linked to metabolic health, with some studies suggesting that a higher ratio of *Firmicutes* to *Bacteroidetes* is associated with obesity and metabolic disorders. However, this relationship remains an area of ongoing research, as other factors such as diet and genetics also influence these microbial populations. [22,23]

As individuals age, their microbiome undergoes notable changes, often characterized by a decline in diversity and shifts in microbial composition. Research has shown that older adults tend to have lower levels of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, which are important for gut health and immune function. At the same time, there is often an increase in potentially harmful taxa, including certain Proteobacteria and Firmicutes, which may contribute to chronic low-grade inflammation, a condition commonly associated with aging, known as "inflammaging." [22,23]

Additionally, aging is often accompanied by lifestyle changes that impact the microbiome. For instance, reduced physical activity, changes in diet (often lower in fiber), and increased use of medications, including antibiotics, can exacerbate the decline in microbial diversity. One example is the rise of *Enterobacteriaceae*, a family within the phylum Proteobacteria, which has been linked to gastrointestinal diseases and systemic inflammation in older populations. [22,23]

Moreover, achieving longevity, potentially living to 100 years or beyond, may be influenced by nurturing a diverse and balanced microbiome. Studies of centenarians, particularly in regions known for longevity such as Okinawa (Japan) and Sardinia (Italy), highlight the role of specific dietary practices and their impact on beneficial microbes. These individuals tend to have higher levels of beneficial microbes, such as *Akkermansia muciniphila* and *Christensenellaceae*, which are thought to support gut integrity and reduce inflammation. [22-24]

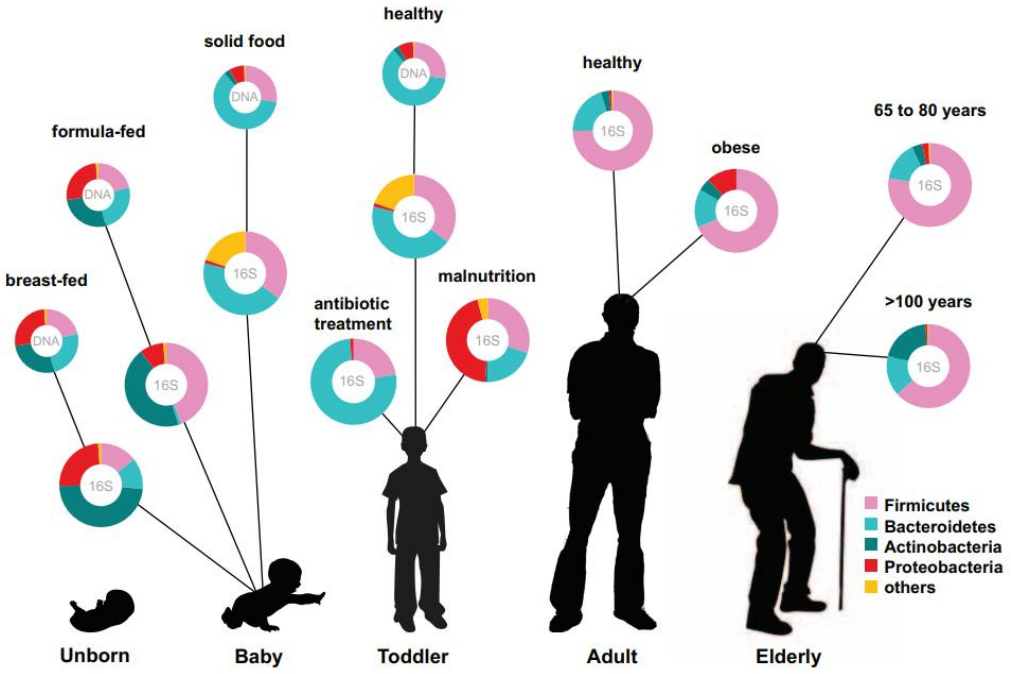

Figure 3.14. Human microbiota: onset and shaping through life stages and perturbations[22]

Throughout life, both intrinsic and extrinsic factors shape microbiome abundance and diversity. Genetics, immune system function, and hormonal changes are intrinsic factors that influence microbial composition. For example, during puberty, hormonal changes can affect

the skin microbiome, leading to conditions such as acne, while immune system maturation during adolescence influences gut microbial stability. [22,23]

Extrinsically, diet remains one of the most significant influences on microbiome composition. In early life, breastfeeding is known to promote the growth of Bifidobacterium, while later in life, diets high in fiber promote microbial diversity and beneficial short-chain fatty acid production. In contrast, diets rich in fat and sugar are associated with a decrease in beneficial microbes and an increase in potentially harmful taxa. [20-23]

Other lifestyle factors, such as exercise, sleep, and stress, also modulate the microbiome. Regular physical activity has been associated with increased microbial diversity, while chronic stress and poor sleep are linked to dysbiosis, or imbalances in the microbiota. [20-23]

The human microbiome undergoes significant changes across different life stages, reflecting the complex interplay between genetic, environmental, and lifestyle factors. From the early colonization in infancy to the declining diversity seen in older adults, the composition of the microbiome has profound implications for health and disease. This early microbial community is relatively simple but rapidly diversifies as the child grows and encounters new environments. In adulthood, the microbiome typically stabilizes, reaching a state of greater diversity and functional complexity, which supports metabolic processes, immune modulation, and resistance to pathogens.

However, as individuals age, there is often a gradual reduction in microbial diversity, accompanied by shifts in the abundance of certain microbial groups.

Table 3.1. Microbiota variations within individuals[23]

		Gut Microbiota Abundance							Bacteria Diversity
		Actinobacteria	Bacteroidetes	Firmicutes	Proteobacteria	Fusobacteria	Verrucomicrobia	Euryarchaeota	
Anatomical part of gut tract	Small intestine			*Lactobacillus*	*Enterobacteriaceae* *				
	Colon		*Bacteroidaceae* * *Prevotellaceae* * *Rikenellaceae* *	*Lachnospiraceae* * *Ruminococcaceae* *					
Gestational age	Preterm birth (<37 weeks of gestation)	*Bifidobacterium* spp.↓ *Atopobium* spp.↓	Bacteroides *↓ (non-secretor mothers)	Firmicutes *↓ (non-secretor mothers) *Lactobacillus*↑ *Ruminococcus* spp. *Lachnospiraceae* * *Peptostreptococcaceae* * *Clostridiaceae* *	*Enterobacteriaceae* *↑ *Enterococcus* spp.↑				↓
	Full-term birth	*Bifidobacterium* spp.↑	Bacteroidetes *↑	*Ruminococcus* spp. *Lachnospiraceae* * *Peptostreptococcaceae* * *Clostridiaceae* *	*Enterobacteriaceae* *				↑
Type of delivery	Vaginal delivery	*Bifidobacterium* spp.↑ *Bifidobacterium longum*↑ *Bifidobacterium catenulatum*↑	*Prevotella*↑ *Bacteroides fragilis*↑	*Lactobacillus*↑ *Staphylococcus*↑ *Streptococcus* ↑	*Escherichia*↑	*Sneathia*↑			↑
	C-section	*Corynebacterium*↑ *Propionibacterium*↑	*Bacteroides* *↓	*Staphylococcus*↑	*Escherichia*↓ *Shigella*↓				↓
Methods of (milk) feedings	Breast milk	*Bifidobacterium*↑↑		*Lactobacillus*↑ *Staphylococcus* ↑	*Enterococcus*↑				↑
	Artificial milk	*Bifidobacterium*↑	*Bacteroides*↑	*Clostridium*↑ *Clostridium difficile*↑ *Lactobacillus*↑	*Escherichia*↑				↓
	Introduction of solid food	*Bifidobacterium*↑	Bacteroidetes *↑ *Bacteroides*↑	Firmicutes *↑ Lactobacilli↑ *Clostridium coccoides*↑					↑
Human age	Childhood (first year of life)	*Bifidobacterium*	*Bacteroides*	*Veillonella* *C. coccoides* *C. botulinum*			*Akkermansia muciniphila*		↑
	2–3 years old to adult	*Bifidobacteriaceae* * *Coriobacteriaceae* *	*Bacteroidaceae* * *Prevotellaceae* * *Rikenellaceae* *	*Lachnospiraceae* *Ruminococcaceae*	Proteobacteria *	*Fusobacteria* *	*Akkermansia muciniphila*	*Methanobrevibacter smithii*	↑
	Over 70	*Bifidobacteriaceae*↓		*Clostridium* *↓	Proteobacteria *↑				↓

These age-related changes can influence susceptibility to infections, chronic inflammation, and other age-associated health conditions. This evolving microbial ecosystem underscores the intricate relationship between the microbiome and human health across the lifespan.

Understanding these age-specific microbiome shifts, and their broader health implications is crucial for developing targeted interventions, such as prebiotics, probiotics, and dietary modifications, to support microbial health.

At this chapter we highlight the dynamic nature of microbial communities across the lifespan, providing a foundation for further exploration into the relationship between microbiome composition and health at different stages of life.

3.9 Different Types of Microbiomes According to Body Sites

The human body is home to a complex and diverse ecosystem of microorganisms collectively referred to as the microbiome. These microbial communities—including bacteria, archaea, fungi, and viruses— inhabit various body sites, each with its own unique composition and functions. (Figure 3.15) Among the most well-studied microbiomes are the gut, oral, skin, lung, vaginal, ear, eye microbiomes and urinary tract microbiome (Figure 3.16- Figure 3.17) which play critical roles in maintaining health and preventing disease. Below we will explore the diversity of microbiomes across different anatomical regions, their specific functions, and their influence on human health. It is crucial to acknowledge the latest biotechnology's involvement when analysing the human microbiome. Its identification is possible with the latest techniques of metatranscriptomic, metagenomic, metabolomic. [25]

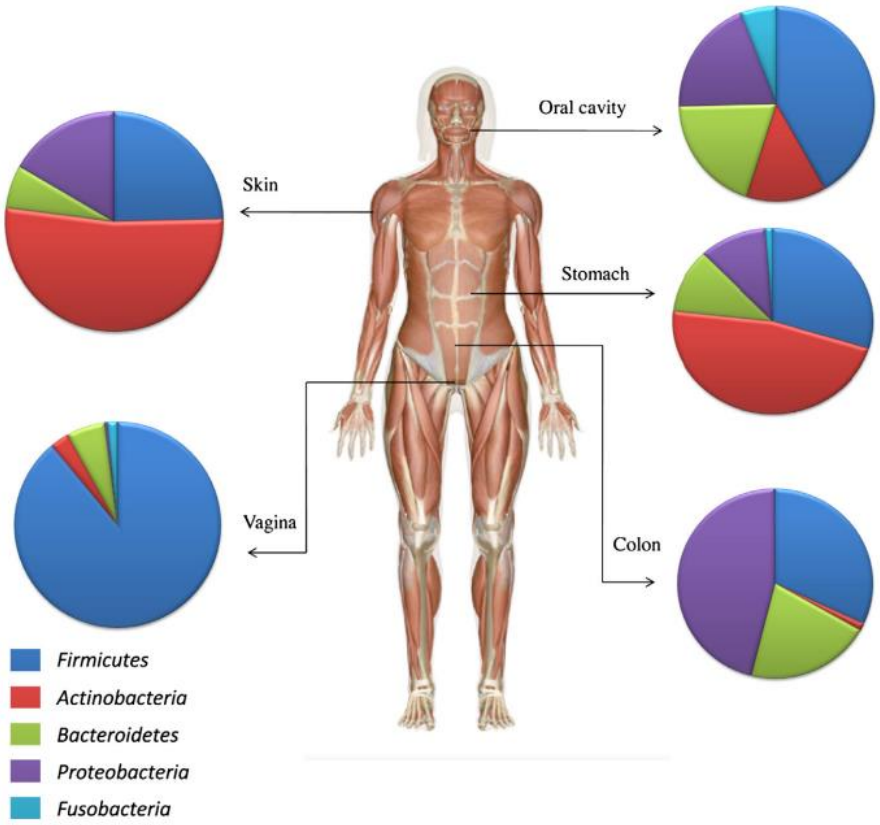

Figure 3.15 Distribution of bacterial phyla in human body sites [27]

3.9.1 Skin Microbiome

The skin microbiome consists of a diverse community of bacteria, fungi, viruses, and mites that reside on the skin's surface. Actinobacteria is the most dominant phyla of the skin. This microbiome acts as a first line of defense against external pathogens and plays a role in skin health and disease. [25-27]

The composition of the skin microbiome varies by body site due to differences in moisture, pH, and sebaceous gland activity. Dominant microorganism genera include *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*, along with *Micrococcus, Brevibacterium*, *Malassezia, Actinobacter, Dermobacter*. Different regions of the skin consist of different microorganisms (Table 3.2). The human skin sites can be categorized into 3 types; it could be sebaceous (oily) (face, chest and back), moist (bend of elbow, back of knee and groin) or dry (volar forearm and palm) (Figure 3.18) [25-30]. The environment of these skin sites is affected by structures such as sweat glands, hair follicles, and sebaceous glands. [25-29]

The most common colonies of the moist areas in skin such as the inner elbow or the gluteal cease are *Staphylococcus spp*. and *Corynebacterium spp.* The fungal population existing in the central body sites is predominantly *Malassezia* and whereas in the foot the predominant fungal species are *Apergillus spp., Rhodotorula spp., Cryptococcus spp., Epicoccum spp.* It is important to understand the composition of the microbiota at different sites to explore the aetiology of common skin disorders, as certain conditions may show preferences for specific skin locations. For example, eczema tends to affect the inside of the elbow, while psoriasis commonly affects the outside (further pathological interactions with the skin microbiome would be explained in the next chapters). Content wise the skin is relatively deficient in resources (in

contrast to the nutrient-dense environment of the gastrointestinal tract), providing primarily basic proteins and lipids. The skin microbiota has developed specialized adaptations to survive under these conditions, characterized by a cool, acidic, and desiccated milieu. Microbes exploit the available substrates in sweat, sebum, and the stratum corneum. For example, *Cutibacterium acnes* (formerly *Propionibacterium acnes*), a facultative anaerobe, thrives within the anaerobic sebaceous glands by utilizing proteases to cleave arginine from skin proteins and lipases to hydrolyze triglycerides in sebum, releasing free fatty acids that facilitate bacterial adhesion. Studies have demonstrated a positive correlation between sebum production on the cheek and the abundance of *Propionibacterium spp*. Notably, in mammals such as mice, rats, and dogs, which produce lower levels of triglyceride-rich sebum, *C. acnces* exhibits reduced growth and is consequently less abundant.[29]

Lipid-dependent species such as *Malassezia* and *Corynebacterium* also thrive in this lipid-rich environment. These species are auxotrophic for lipids, relying on external lipid sources for growth. *Corynebacterium spp*. utilize these lipids to synthesize corynemycolic acids, which are essential for coating their cell surface. Consistent with the lipid-dominant, carbohydrate-limited environment of the skin, the genomes of *Malassezia* species are enriched in lipase-encoding genes and display a reduction in genes responsible for carbohydrate metabolism when compared to other sequenced fungi. [29]

The latest microorganism for the skin microbiome is *Staphylococcus spp.* that can be resistant to the very salty environment of the skin. This particular characteristic is called to be halotolerant. They also use urea as source of nitrogen. In order to enhance colonization, certain *Staphylococcus species* are capable of producing adhesion molecules that facilitate their attachment to the skin surface, as well as proteases that release nutrients from the stratum corneum. The composition of skin microbial communities in adults remains stable, as observed

over a period of at least two years. The skin, as a whole, hosts a diverse community of microorganisms, each equipped with specialized adaptations to thrive in this environment. [25-29]

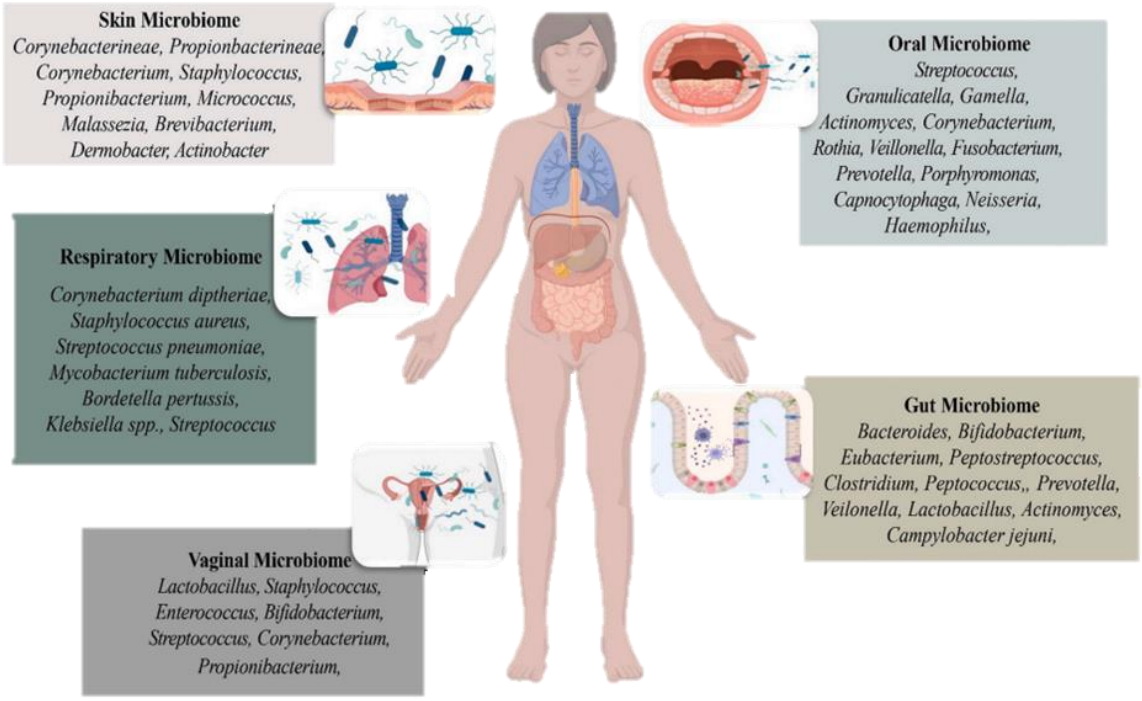

Figure 3.16 Overview of the human microbiome localized in different body tissues.[27]

*Staphylococcus epidermidi*s and *Propionibacterium acnes* playing key roles in skin homeostasis. These bacteria help regulate the skin's immune response and prevent colonization by harmful pathogens. A balanced skin microbiome protects against skin infections by competing with pathogenic bacteria and modulating the skin's immune system. Dysbiosis in the skin microbiome has been linked to conditions such as acne, atopic dermatitis, and psoriasis. For example, an overgrowth of *Propionibacterium acnes* is associated with acne, while reduced microbial diversity on the skin is linked to atopic dermatitis.[27-30]

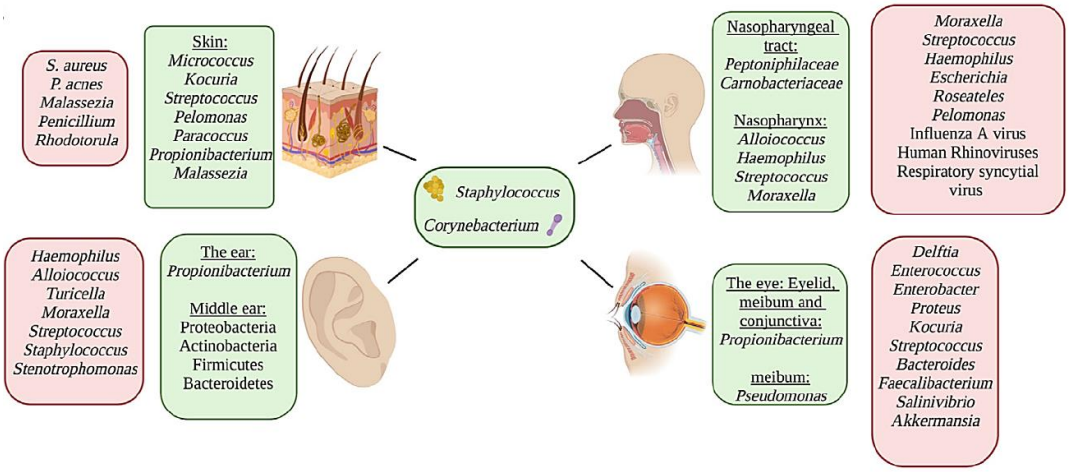

Figure 3.17. Characteristic microbiome of the skin, ear, eyes and the nasopharyngeal tract. Homeostatic microbiomes are identified in green, and microorganisms that increase in abundance during dysbiosis are identified in red.[28]

Table 3.2. Microorganisms exists on different region of the skin [25]

Region	Predominant organism
Scalp	*Staphylococci* (*S. captis, S. epidermidis* etc.), *Propionibacterium acne, P. granulosum, P. avedum, Malassezia* spp.
Toe interspace	*Staphylococcus epidermidis, S. haemolyticus, S. cohnii, S. hominis, S. warneri, Micrococcus* spp., *Malassezia* spp.
Perineum	*P. acne, P. granulosum, P. avedum, S. epidermidis, S. hominis, S. aureus, Coronybacterium minutissimum, C. xerosis, C. jeikeium, Malassezia* spp., *Strepyococci, E. coli*
Axillae	*P. acne, P. avidum, P. granulosum, S. aureus, S epidermidis, S. saprophyticus, C. xerosis, C. minutissimum*, Gram-negative rods (*E. coli, Klebsiella, Proteus, Enterobacter* spp, *Actinobacter* spp)
Sole of the foot	*S. epidermidis, S. hominis, S. haemolyticus, S. cohnii, S. warneri, Malassezia* spp, *Micrococcus* spp, aerobic coryneforms, Gram-negative organism
Forearm and leg	*Staphylococci* (*S. haemolyticus, S. epidermidis, S. aureus, S. hominis), coryneform* and *propionibacteria*
Hands	*S. aureus, S. epidermidis, S. hominis, C. xerosis, C. minutissimum,* yeast and other fungi (*Candida parapsilosis, Rhodotorula rubra*), Gram-negative *bacilli* (*Pseudomonas* spp, *Enterobacter* spp)
Outer ear	*S. auricularis, S. epidermidis, S. captis, S. aureus, S. caprae, Brevibacterium* spp, *Turicella otitidis, Alloiococcus otitis*

Malassesia and *Acremonium* dominantly present during atopic dermatitis. Restoration of a healthy skin microbiome through topical probiotics and other therapies is being explored as a treatment for these conditions. In a recent study which is the first study that used autologous

transplant of microorganisms to the skin, several coagulase-negative *Staphylococcus species*, including *S. epidermidis* and *S. hominis,* were found to produce novel antibiotics that could act synergistically with the human antimicrobial peptide LL-37, effectively inhibiting the growth of S. aureus. Individuals with atopic dermatitis, who are often colonized by S. aureus, exhibited a depletion of antibiotic-producing strains. Furthermore, the topical application of these antimicrobial-producing strains significantly reduced S. aureus colonization in a small cohort of individuals with atopic dermatitis, highlighting the potential for a probiotic therapeutic approach[29] (A detailed therapeutic options of the microbiome in atopic dermatitis would be elaborated in the next chapters)

3.9.2 Oral Microbiome

The oral microbiome refers to the combined genetic material of the microorganisms that inhabit the oral cavity. The oral microbiome consists of more than 700 bacterial species residing in various niches such as the tongue, teeth, gingiva, and saliva. It plays a key role in maintaining oral health and serves as a critical barrier against pathogens. [31]

Once all teeth have erupted, additional surfaces become available for microbial colonization. The formation of gingival crevices facilitates the establishment of periodontal microorganisms. Plaque tends to accumulate in various locations on the teeth, including smooth surfaces as well as pits and fissures, allowing for the development of distinct microbial communities. This process promotes both high species diversity and microbial succession. As individuals age and

lose their teeth, the microbial flora increasingly resembles that of a child prior to the eruption of teeth.[31]

The oral cavity is mainly the most studied microbiomes, and it is home to a balanced microbial ecosystem, where commensal bacteria such as *Streptococcus*, *Actinomyces*, and *Veillonella* contribute to oral health by preventing the overgrowth of pathogenic microorganisms. These commensals inhibit pathogenic bacteria through competitive exclusion, the production of antimicrobial peptides, and modulation of the local immune response. [31]

The oral cavity hosts a diverse array of species belonging to 185 genera and 12 phyla, with roughly 54% being formally described, 14% unnamed yet cultivated, and 32% recognized solely as uncultivated phylotypes. The identified phyla include *Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, SR1*, *Synergistetes, Saccharibacteria (TM7),* and *Gracilibacteria* (GN02). Within healthy individuals, a core oral microbial community is maintained at the genus level, though diversity is both individual- and site-specific. The tongue, characterized by numerous papillae and fewer anaerobic sites, supports a rich microflora, including both aerobic and anaerobic organisms. In contrast, the buccal and palatal mucosae exhibit lower microbial diversity.[31]

The composition and activity of the oral microbiome can undergo significant and rapid changes both spatially and temporally, demonstrating a dynamic relationship with the host throughout development. These complex, nonequilibrium dynamics arise from various factors, including the timing of dietary intake and host activity, pH fluctuations, inter-bacterial interactions, and over longer periods, genetic mutations and horizontal gene transfer that can impart new traits to microbial strains.[31]

Microorganisms in the oral cavity interact in a symbiotic manner that provides mutual benefits. Commensal bacteria contribute to oral health by inhibiting the adhesion of pathogenic species to the mucosal surfaces, effectively preventing harm. Pathogens typically become problematic only when they manage to breach the protective layer established by these commensal populations, resulting in infection and disease.[31,32]

Oral dysbiosis can result in diseases like dental caries, periodontal disease, and oral thrush. Pathogenic bacteria such as *Porphyromonas gingivalis* and *Treponema denticola* contribute to periodontal disease, leading to inflammation and tissue destruction. Moreover, oral dysbiosis is associated with systemic conditions like cardiovascular disease, diabetes, and adverse pregnancy outcomes, as oral bacteria can translocate into the bloodstream and exacerbate systemic inflammation.[31,32]

The oral microbial communities within the human body contribute to essential physiological, metabolic, and immunological functions. These include aiding in food digestion and nutrient absorption, generating energy, supporting the differentiation and maturation of the host's mucosal tissues and immune system, regulating fat storage and metabolism, detoxifying environmental chemicals, and maintaining the barrier function of the skin and mucosal surfaces. Additionally, they play a crucial role in sustaining immune homeostasis by balancing pro-inflammatory and anti-inflammatory responses, promoting beneficial microorganisms (colonization resistance), and preventing the invasion and proliferation of pathogens. [31]

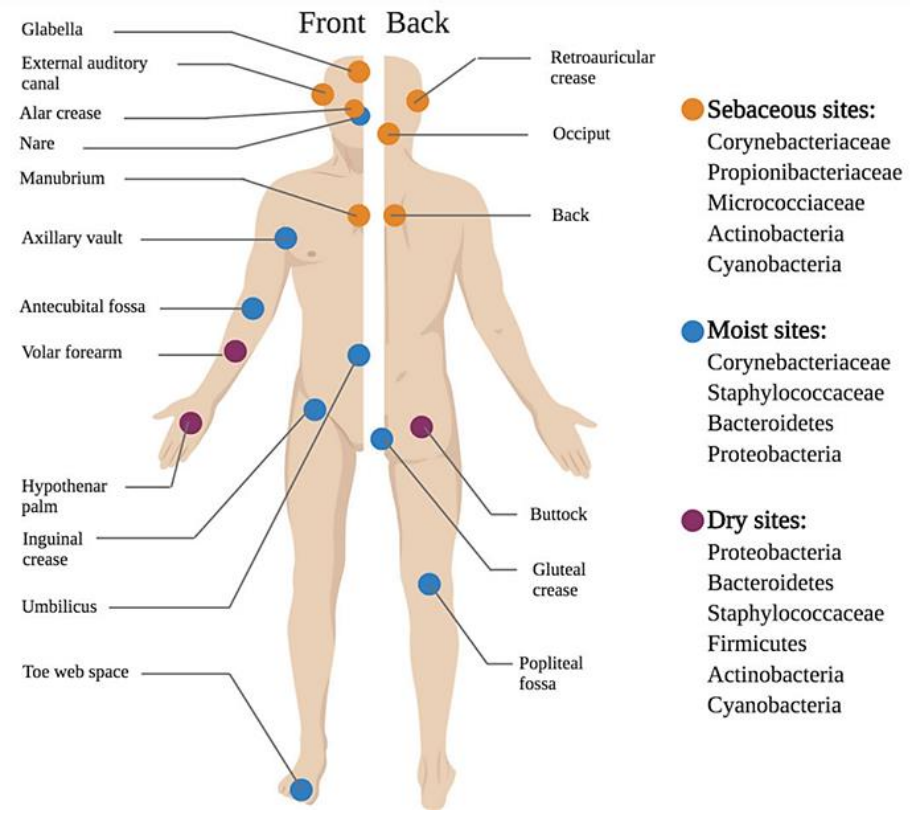

Figure 3.18 Composition of the skin microbiome via different types [30]

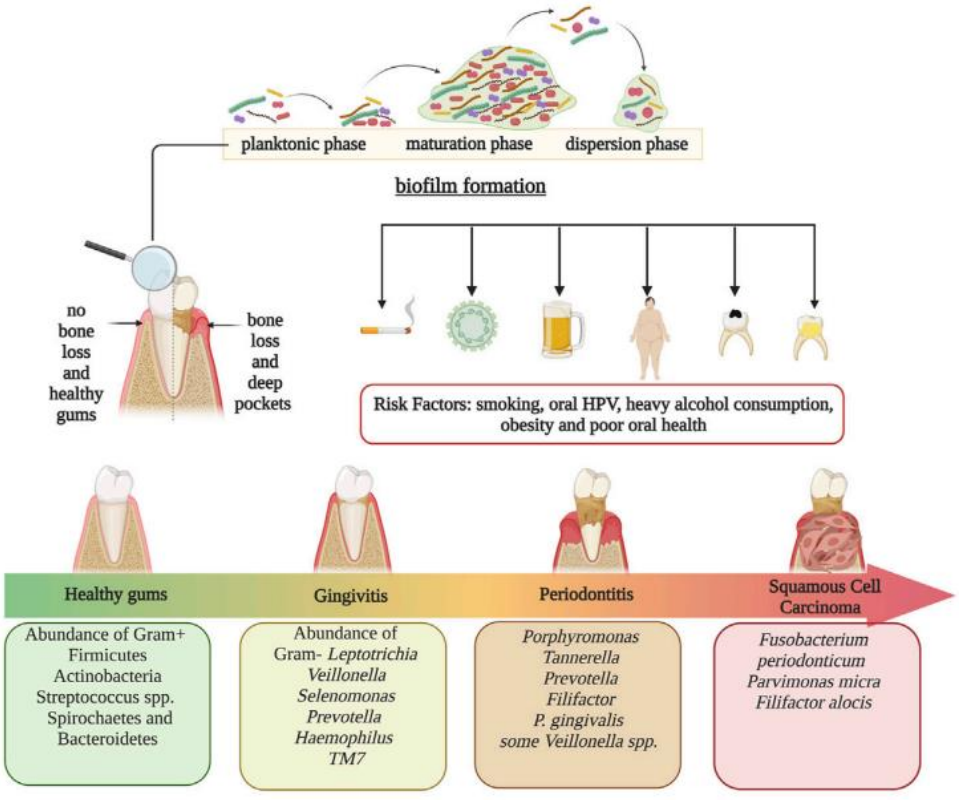

Figure 3.19 Composition of the oral microbiome along with the biofilm formation [28]

3.9.3 Gut Microbiome

The gut microbiome is the most densely populated and diverse microbial community in the human body, with over 100 trillion microorganisms primarily composed of bacteria from the phyla *Firmicutes* and *Bacteroidetes*. This highly complex microbiome secondly composed of bacteria from the phyla *Actinobacteria* and *Proteobacteria* (Fig 3.20). [28,33] The colonization of the organisms in the gut differs since the gastrointestinal tract starts from the mouth and ends with anus, therefore each digestive organ will contribute to a specific environment. There are multiple factors; such as pH, infant feeding method, birthing process, diet, geography, lifecycle stages, stress or biochemical features that plays a role in these different sections (Figure 3.21). [30-34]

In the small intestine, a gradient in microbial concentration is observed, where microbial counts in duodenal samples are approximately 1,000 times lower than in oral samples, although they share some similar taxa. Consequently, the small intestine gradually accumulates microbial populations, ranging from thousands to hundreds of millions of cells per gram of contents, with a predominance of partially oxygen-tolerant phyla, such as *Firmicutes* and *Proteobacteria*. The microbial density peaks in the colon, which harbors up to 100 billion cells per gram and allows these communities to persist for extended periods due to a much slower transit time compared to the small intestine. In turn, the colon's microbiome is characterized by anaerobic bacteria, encompassing thousands of species and millions of genes, primarily from the phyla *Firmicutes* (notably *Ruminococcaceae* and *Lachnospiraceae*), *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (such as *Akkermansia*).

This microbial mass, excreted as feces, forms what is generally known as the gut microbiome, which is highly adaptable to dietary and medicinal changes and has been associated with

numerous health conditions. The gut microbiome also serves as the foundation for fecal microbiota transplantation (FMT), which has proven effective in treating recurrent *Clostridioides difficile* infections and other conditions. Notably, humans can live without a colon but cannot survive without the small intestine, which has the largest mucosal surface in the body, where nutrient absorption occurs, and contains the majority of gut receptors, immune cells, and neurons involved in critical interactions with microbes.[35]

The gut microbiome is involved in critical physiological processes, including the digestion of complex carbohydrates, the production of short-chain fatty acids (SCFAs), and the modulation of the immune system.[33-35]

The small intestine is highly expert on breaking down the nutrients, emulsification, and absorption, allowing minimal nutrients to bypass digestion. Under typical conditions, for instance, less than 4-5 grams of fat per day will reach the colon. A similar process applies to simple carbohydrates, which are broken down into glucoses, and most proteins, which are converted into amino acids, although some protein may pass into the colon depending on dietary intake. In contrast, complex carbohydrates, like dietary fibers, are indigestible due to the lack of specific enzymes, allowing them to pass through the small intestine undigested. These fibers, however, serve as an energy source for certain bacteria residing in the colon. Various gut microbes play a role in breaking down these indigestible carbohydrates into short-SCFAs such as acetate, butyrate, and propionate. These SCFAs are chemically well-defined, and their health benefits are well-documented. They influence numerous metabolic pathways, not only within the gut but also in distant organs, including the liver, adipose tissue, muscles, and brain. Today, these microbial metabolites are recognized for their broad physiological effects, impacting processes such as energy balance, glucose and lipid metabolism, inflammation, immunity, and even cancer progression. [35]

Key bacterial species in the gut include Bacteroides, which are responsible for breaking down complex polysaccharides into simpler molecules that can be absorbed by the body. *Firmicutes*, such as *Faecalibacterium prausnitzii*, produce butyrate, an SCFA that serves as an energy source for colonocytes and has anti-inflammatory properties. These bacteria maintain the integrity of the gut barrier and prevent the colonization of pathogenic bacteria. [33-35]

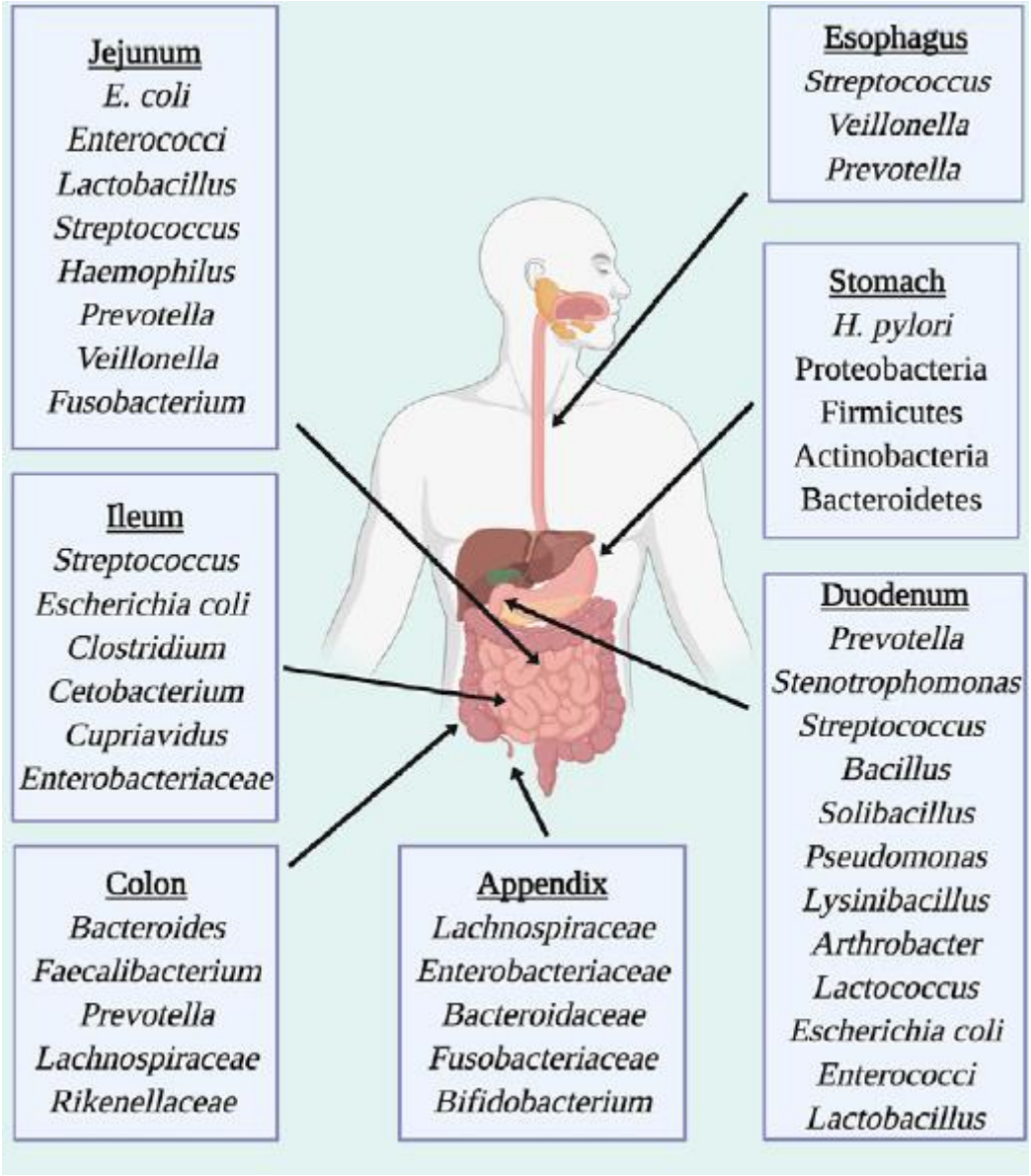

Figure 3.20 Complex composition the gut microbiome in different sections of gastrointestinal tract [28]

A healthy gut microbiome promotes immune system development, protects against pathogenic infections, and contributes to metabolic homeostasis. Conversely, dysbiosis—an imbalance in gut microbial composition—has been linked to a variety of diseases, including inflammatory bowel disease (IBD), obesity, type 2 diabetes, and colorectal cancer. The gut microbiome also influences the gut-brain axis, affecting mental health and neurodegenerative diseases.[33-35]

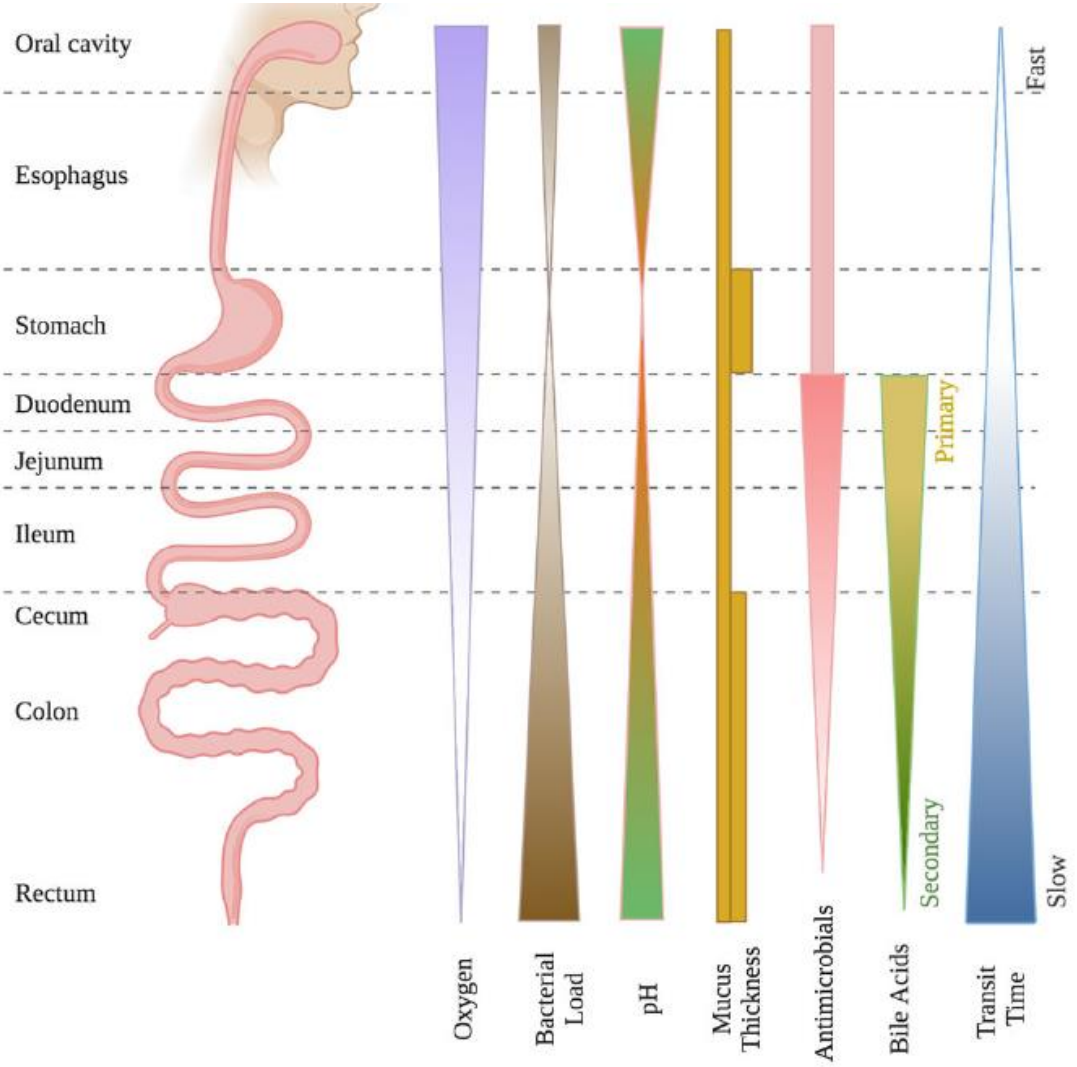

Figure 3.21 Factors affecting the gut microbiome in different sections of gastrointestinal tract [30]

3.9.4 Respiratory Tract (Lung) Microbiome

The lung microbiome was once thought to be sterile, but recent research has revealed that the respiratory tract harbors a distinct and dynamic microbial community. The lung microbiome plays a crucial role in maintaining pulmonary health by regulating immune responses and defending against respiratory pathogens.[36,37]

The lung microbiome is less dense than the gut microbiome, but it contains bacterial communities similar to those found in the upper respiratory tract, including genera like *Streptococcus, Prevotella*, and *Veillonella.* (Figure 3.22) These bacteria help maintain the homeostasis of the lung environment and modulate immune responses to inhaled pathogens and allergens.[36]

A balanced lung microbiome is crucial for preventing respiratory infections and maintaining healthy lung function. In healthy individuals, small amounts of saliva containing bacteria are aspirated into the lungs. However, the extent of bacterial replication within healthy airways is not fully understood, as environmental factors in the alveoli—such as elevated surfactant levels, oxygen concentration, and mucosal pH—create unique conditions within the lungs. The lungs work to maintain a low microbial load to optimize gas exchange efficiency. Increased bacterial biomass in the airways is linked to imbalanced airway immunity, progressive tissue damage, and inflammation. Bacteria are removed from the airways through mechanisms like coughing, mucociliary clearance, and immune defenses. Thus, the lung microbiome remains a dynamic system, with microbial communities constantly turning over and being replenished by sources from the oropharynx and environment. In healthy lungs, microbial biomass remains relatively low, at about up to 10^5 bacteria per gram of tissue, which is much less dense than the microbial population in the lower gastrointestinal (GI) tract, where levels reach up to 10^{12} bacteria per

gram. Although the lungs and GI tract both originate embryologically from mucosa-lined luminal tissues, they differ significantly in micro-anatomical structures and environmental conditions. For instance, the movement of microorganisms in the GI tract is one way, moving from the mouth to the anus under normal conditions, unless disrupted by vomiting or acid reflux. Microbes that enter the digestive tract orally must first withstand the stomach's acidic pH and then adapt to the alkaline environment of the duodenum before reaching the cecum. [37-40]

Conversely, the lungs have a bidirectional flow of air, mucus, and microbes due to the respiratory process. This bidirectionality creates a dynamic and transient lung microbiome, contrasting with the more stable microbial populations of the GI tract. Unlike the consistent internal temperature of 37°C in the GI tract, the respiratory tract features a temperature gradient from ambient levels at the airway openings to core body temperature within the alveoli. Additionally, the lungs are rich in oxygen under healthy conditions, creating an aerobic environment, whereas the GI tract is largely anaerobic. The mucus lining of the trachea and bronchi in the lungs is somewhat comparable to that of the GI tract, but the alveoli, which are primarily covered with lipid-rich surfactant, exhibit bacteriostatic properties against certain bacterial species.[38-40]

Further differences exist in immune interactions between the two systems. While the GI tract has higher concentrations of luminal IgA, the lung environment relies more on extraluminal interactions, with alveolar macrophages playing a prominent role in host defense. These diverse environmental factors and immune dynamics lead to distinct microbial communities between the lungs and GI tract, reflecting each organ’s unique physiological demands and microbial management. [38-40]

This continuous interaction is vital for modulating immune responses in the lungs. In disease states, numerous pre-clinical and clinical human studies have shown that disruptions in lung microbiota are associated with changes in alveolar immunity and clinical outcome. [36-38]

Dysbiosis in the lung microbiome has been implicated in chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis. In these conditions, pathogenic bacteria like Pseudomonas aeruginosa and *Staphylococcus aureus* dominate the microbial landscape, exacerbating inflammation and tissue damage.[36]

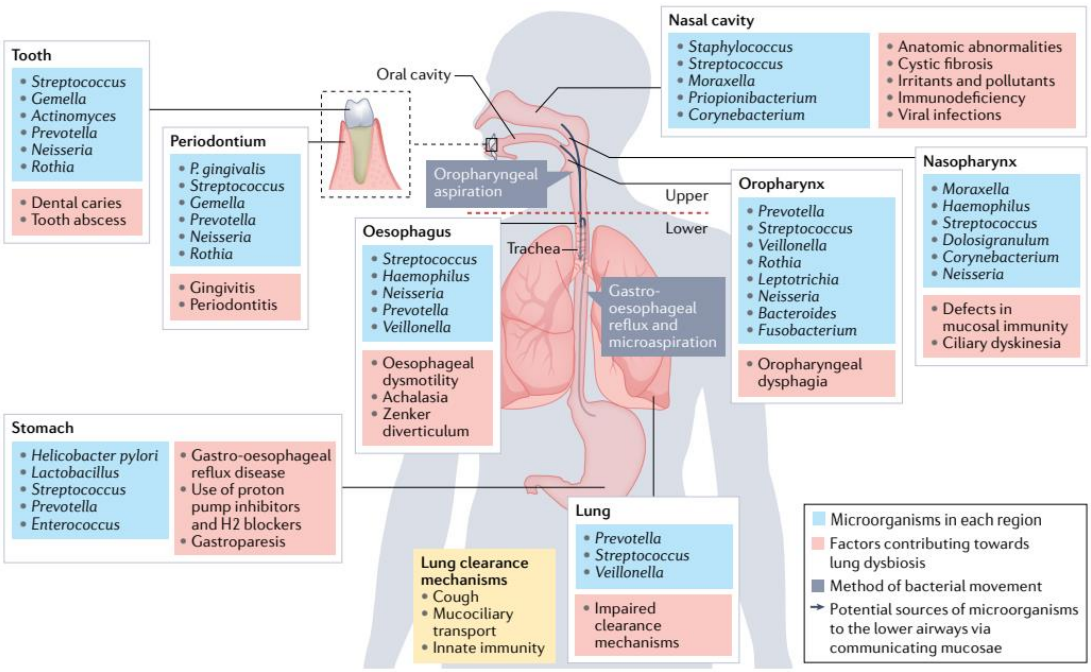

Figure 3.22 Healthy microbiota of the respiratory and proximal gastrointestinal tracts [36]

3.9.5 Vaginal Microbiome

The vaginal microbiome is distinct from other body microbiomes in that it is typically dominated by a small number of bacterial species, primarily from the *Lactobacillus* genus. These bacteria are critical for maintaining vaginal health and protecting against infections. [41]

The predominant species in a healthy vaginal microbiome are Lactobacillus crispatus, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus iners*. These bacteria produce lactic acid, which lowers the vaginal pH to a protective range of 3.5 to 4.5, preventing the overgrowth of harmful pathogens such as *Gardnerella vaginalis* and *Candida albicans*. In addition to lactic acid, *Lactobacillus* species also produce bacteriocins and hydrogen peroxide, further inhibiting pathogenic microorganisms via forming a defense barrier. Some of the studies shown a reverse effect for hydrogen peroxide, when found in great amounts, it could be having an antimicrobial effect for Lactobacillus spp. instead of pathogenic microorganisms. [41,42]

A balanced vaginal microbiome is essential for reproductive health, as it helps prevent bacterial vaginosis (BV), yeast infections, and sexually transmitted infections (STIs). Vaginal dysbiosis, characterized by a reduction in Lactobacillus and an increase in anaerobic bacteria, is associated with an increased risk of BV, STIs, and adverse pregnancy outcomes such as preterm birth and miscarriage.[43]

3.9.6 Urinary Tract Microbiome

The concept of a microbiome in the urinary tract challenges the long-held belief that urine in healthy individuals is microorganism-free. Traditionally, the detection of microorganisms in urine was exclusively associated with infections, specifically urinary tract infections (UTIs). However, with advances in culture-independent molecular techniques such as 16S rRNA gene

sequencing and next-generation sequencing (NGS), we now understand that a diverse microbial community resides in the urinary tract, even in the absence of clinical infection. This microbial community plays a crucial role in maintaining urinary tract health, preventing pathogen colonization, and modulating the host immune response.[44]

The urinary tract microbiome (UTM) is less studied compared to the microbiomes of other body sites like the gut or skin, but it has become clear that it contributes to both health and disease. Recent research indicates that the urinary tract contains its own distinct microbial community, known as the urinary microbiota, which differs significantly from the microbial populations found in the gut and vaginal environments. Unlike these other sites, the urinary microbiota is adapted to the unique conditions of the urinary tract, such as its pH, flow dynamics, and epithelial surface, all of which contribute to shaping its specialized microbial composition. The diverse microbial community residing within the urinary tract, composed primarily of bacteria but also including fungi and viruses. This microbiome differs by sex and age, with women typically harboring *Lactobacillus* species that play a protective role, while men tend to have more diverse bacteria like *Corynebacterium* and *Streptococcus*. Age-related changes, particularly in postmenopausal women, can result in decreased diversity and protective bacteria, increasing susceptibility to urinary conditions. In the urethra, the microbiota primarily comprises members of the *Lactobacillaceae* family, coagulase-negative Staphylococcus, non-hemolytic *Streptococcus spp*. (such as viridans group), *Lactobacillus spp., Mycoplasma spp*., *Coccobacilli*, *Diphtheroids, non-pathogenic Neisseria*, some anaerobes, and occasionally Saccharomyces. In the bladder, symbiotic bacteria, including species from *Lactobacillaceae* such as *Lactobacillus*, along with *Staphylococcus* and *Gardnerella*, are thought to play a protective role by maintaining urinary system homeostasis and limiting pathogen growth. Ongoing research continues to shed light on the diversity and composition of

urinary microbiota communities, exploring their influence on health and the shifts that occur between states of microbial balance (eubiosis) and imbalance (dysbiosis).[44-46]

The UTM functions primarily as a defense against pathogens by maintaining microbial balance and preventing harmful bacteria from colonizing. Additionally, it plays a role in modulating immune responses, helping to prevent chronic inflammatory conditions in the bladder. When this microbial balance is disrupted, dysbiosis can occur, contributing to conditions like recurrent urinary tract infections (UTIs), interstitial cystitis, and potentially bladder cancer. The relationship between dysbiosis and disease underscores the importance of maintaining a healthy UTM for urinary health.[44]

Emerging research suggests that the UTM holds potential for both diagnostics and therapeutic interventions. Changes in microbial composition could be used as biomarkers for urinary diseases, and therapies such as probiotics and bacteriophage treatments may offer promising alternatives to traditional antibiotics. As research continues to uncover the significance of the urinary microbiome, its role in maintaining urinary health and its implications in disease will likely become central to future medical practices.[44-46]

3.9.6 Eye Microbiome

The eye microbiome primarily inhabits the conjunctiva and ocular surface, consisting of bacterial species such as *Corynebacterium, Propionibacterium, Streptococcus, Staphylococcus*. These microbes play a critical role in protecting the eye from pathogenic infections like conjunctivitis and keratitis by forming a barrier and interacting with the immune system. The delicate microbial ecosystem of the eye also maintains a balance that prevents chronic inflammatory conditions like blepharitis and helps support overall ocular surface health.[47,48]

When this balance is disrupted—due to factors like contact lens use, environmental stressors, or antibiotic treatments—dysbiosis can lead to increased susceptibility to infections or conditions like dry eye syndrome. Infections like bacterial conjunctivitis often involve the overgrowth of harmful bacteria in a compromised microbiome. Maintaining a healthy eye microbiome is essential for reducing inflammation, promoting healing, and preventing recurrent infections. [47-50]

Therapeutic interventions, including the development of microbiome-targeted treatments like probiotics or bacteriophage therapy, are emerging as potential solutions for managing eye diseases. These treatments aim to restore microbial balance and enhance the eye's natural defense mechanisms, offering new approaches to treat infections and inflammatory conditions while minimizing the adverse effects of conventional therapies. [47-50]

3.9.7 Ear Microbiome

The ear microbiome is primarily located in the external auditory canal, with bacteria such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* forming its core. These bacteria maintain a delicate balance, preventing the overgrowth of pathogens that could lead to conditions like otitis externa, commonly known as "swimmer's ear." Environmental factors, such as moisture or excessive cleaning, can disturb this microbial balance, leading to infections or chronic inflammation. The presence of these beneficial microbes is crucial for maintaining the ear's health by inhibiting pathogen colonization and supporting immune function.[51,52]

Dysbiosis in the ear microbiome, often triggered by antibiotics, injury, or environmental changes, can result in recurrent ear infections or chronic conditions. Children, in particular, are prone to otitis media, a middle ear infection, where microbial shifts contribute to persistent

inflammation. In adults, disruptions in the ear microbiome are associated with conditions like chronic otitis externa. Maintaining microbial diversity and balance in the ear is critical for preventing these infections and managing inflammation. [51,52]

The study of the ear microbiome offers new possibilities for non-invasive treatments, such as probiotics, which could restore microbial equilibrium and reduce the recurrence of infections. As research progresses, a better understanding of how the ear's microbial community interacts with pathogens may lead to targeted therapies that enhance ear health while minimizing the need for antibiotics.[51,52]

3.10 Functional Roles of the Microbiome

The human microbiome, which refers to the collective genomes of microorganisms residing in and on the human body, plays a crucial role in numerous biological functions that are vital to maintaining health. These microorganisms—including bacteria, archaea, fungi, and viruses—populate various body sites such as the skin, oral cavity, respiratory tract, gastrointestinal (GI) tract, and reproductive system. The gut and vaginal microbiomes, in particular, have been extensively studied for their roles in immune modulation, metabolic regulation, and pathogen defense. This chapter elaborates on the diverse functions of the microbiome across different body sites, highlighting its role in health, disease, and its therapeutic potential. [25,27,28] A more detailed aspects of the microbiome's therapeutic effects in IMIDs is elaborated in the next chapters.

The gut microbiome is predominantly composed of bacteria from the *Firmicutes* and *Bacteroidetes* phyla, which are essential for digestion, nutrient absorption, and immune system modulation. For example, *Bacteroides thetaiotaomicron* helps break down complex

carbohydrates, producing short-chain fatty acids (SCFAs) such as butyrate, which fuel colon cells and have anti-inflammatory effects. Beneficial bacteria like *Faecalibacterium prausnitzii* produce anti-inflammatory compounds that protect the gut from diseases like inflammatory bowel disease (IBD). [35,53]

In contrast, dysbiosis, or an imbalance in the gut microbiome, can lead to conditions like obesity, type 2 diabetes, and inflammatory diseases. For example, a high ratio of *Firmicutes* to *Bacteroidetes* has been linked to obesity, as certain microbial communities extract more energy from food. [22]

The microbiome and immune system engage in constant communication, with microbes playing a key role in educating and modulating immune responses. From birth, microbes such as *Bifidobacterium* in infants promote the development of immune cells like T regulatory (Treg) cells, which prevent autoimmune reactions. *Lactobacillus reuteri*, for example, can stimulate the production of anti-inflammatory cytokines like IL-10. [22,54]

When the microbial balance is disrupted, the immune system can become hyperactive, leading to chronic inflammation, as seen in conditions like IBD. In such cases, harmful bacteria like *Escherichia coli* promote inflammation, while beneficial bacteria are reduced. [35]

The vaginal microbiome is primarily composed of *Lactobacillus* species, which play a vital role in maintaining reproductive health. Unlike other body sites where microbial diversity indicates health, a healthy vaginal microbiome is dominated by a few species of *Lactobacillus* that produce lactic acid. This acidification (pH between 3.5 and 4.5) creates a hostile environment for pathogenic bacteria and protects against infections such as bacterial vaginosis (BV) and sexually transmitted infections (STIs). [42, 43]

For example, *Lactobacillus crispatus* is known to produce both lactic acid and bacteriocins, antimicrobial peptides that inhibit pathogens like *Gardnerella vaginalis*, the primary culprit in BV. A healthy vaginal microbiome is particularly important during pregnancy, where dysbiosis has been linked to complications like preterm birth and miscarriage. [43]

Hormonal changes, particularly during pregnancy and menopause, also influence the vaginal microbiome. For instance, estrogen promotes *Lactobacillus* growth by increasing glycogen production in vaginal cells, which these bacteria ferment into lactic acid. Postmenopausal women, with lower estrogen levels, often experience a decline in *Lactobacillus* and an increased risk of infections. [42,43]

The gut microbiome also influences host metabolism and energy regulation, playing a significant role in conditions like obesity. Studies have shown that individuals with a higher proportion of *Firmicutes* relative to *Bacteroidetes* tend to extract more calories from the same amount of food, which can contribute to weight gain. This phenomenon has been demonstrated in germ-free mice colonized with microbiota from obese mice, where the recipient mice gained more weight compared to those colonized with microbiota from lean mice, despite being fed the same diet. [22,23,35]

Additionally, bacteria like *Akkermansia muciniphila*, which degrades mucins in the gut, have been linked to improved metabolic health, reduced fat accumulation, and better insulin sensitivity. These findings point to the potential for targeting the microbiome in the management of metabolic disorders. [22, 35]

The gut microbiome also plays a critical role in modulating the gut-brain axis, a bidirectional communication system between the gut and the brain. Certain gut bacteria produce

neurotransmitters and neuromodulators that can influence mental health. For instance, *Bifidobacterium infantis* produces gamma-aminobutyric acid (GABA), which is involved in reducing anxiety and promoting relaxation. [55, 56]

Studies have shown that *Lactobacillus rhamnosus* can reduce anxiety-like behaviors in mice by modulating GABA receptor expression in the brain. Moreover, microbial metabolites like SCFAs can cross the blood-brain barrier and reduce neuroinflammation, which is relevant to neurodegenerative diseases such as Alzheimer's and Parkinson's disease. For example, butyrate, produced by *Clostridium butyricum*, has shown potential in reducing neuroinflammation in animal models of Parkinson's. [55, 57]

Microbiome dysbiosis is closely linked to the development of several diseases. In colorectal cancer (CRC), bacteria like *Fusobacterium nucleatum* have been shown to promote tumorigenesis by inducing pro-inflammatory pathways. Similarly, in metabolic disorders such as type 2 diabetes, individuals often exhibit a dysbiotic microbiome with fewer SCFA-producing bacteria and an increase in opportunistic pathogens like *Proteobacteria*.[23,58]

Conditions such as bacterial vaginosis (BV), recurrent vulvovaginal candidiasis (yeast infections), and persistent human papillomavirus (HPV) infections have also been linked to vaginal microbiome dysbiosis. For instance, lower levels of *Lactobacillus* and higher microbial diversity in the vaginal microbiome have been associated with increased risks of STIs and cervical cancer. [43-45, 59]

Given the crucial role of the microbiome in health, therapies that target the microbiome are gaining attention. Probiotics and prebiotics are commonly used to restore microbial balance. For example, probiotic strains like *Lactobacillus rhamnosus* and *Bifidobacterium* species are

effective in treating bacterial vaginosis and yeast infections by replenishing beneficial microbes. [43-45]

Fecal microbiota transplantation (FMT) is an established therapy for recurrent *Clostridioides difficile* infections, and it is being explored for other conditions like IBD and metabolic syndrome. Similarly, vaginal microbiome transplantation (VMT) is an emerging treatment for recurrent BV, where healthy vaginal fluid is transferred to restore a healthy microbial balance.[34,43,44, 60]

The human microbiome, encompassing the gut, vaginal, and other body sites, is a critical factor in maintaining health. It plays diverse roles in immune regulation, metabolism, neurobehavioral functions, and protection against pathogens. Dysbiosis, or microbial imbalance, contributes to various diseases, from metabolic disorders to infections and cancers. Therapies that target the microbiome, including probiotics, prebiotics, FMT, and emerging treatments like VMT, offer promising strategies for restoring microbial balance and preventing disease. As research progresses, the microbiome's potential in personalized medicine and disease management continues to expand. [22, 34, 60]

3.10.1 The Microbiome and Its Impact on Immune Regulation

The human microbiome, composed of trillions of microorganisms spanning bacteria, fungi, viruses, and archaea, is increasingly recognized as an essential regulator of immune function. As stated above these microbial communities inhabit various body sites, including the gut, skin, lungs, and mucosal surfaces, collectively playing a vital role in shaping the immune system. Far from being passive occupants, these microbes actively interact with the host's immune system, influencing its development, functionality, and responses to external challenges.[61]

The intricate relationship between the microbiome and the immune system is pivotal for maintaining homeostasis. It involves a dynamic balance between tolerance to commensal organisms and robust defense mechanisms against pathogens. Disruptions in this relationship, known as dysbiosis, can lead to immune dysregulation, manifesting as autoimmune diseases, allergies, and chronic inflammatory conditions. The immune system plays a crucial role in regulating both healthy and imbalanced microbiota. This has been demonstrated through research on bacterial colonization in wild-type animals and mice deficient in pattern recognition receptors (PRRs). Mice lacking PRRs, such as Toll-like receptors (TLRs) or nucleotide-binding oligomerization domain-like receptors (NODs), exhibit variations in their intestinal microbiota composition and reduced production of antimicrobial peptides like defensins. Furthermore, studies show that germ-free mice have lower levels of immunoglobulin A (IgA) and fewer B cells, highlighting the interaction between the host's immune system and its microbiota. Essentially, the ability of host cells to recognize microorganisms influences whether an inflammatory response is triggered, or homeostasis is maintained. Additionally, immunodeficient hosts have been found to harbor a dysbiotic microbiota. [61-65] This chapter explores the complex crosstalk between the microbiome and host immune system, as well as the detrimental consequences of dysbiosis on immune regulation and disease development.

3.10.2 Microbiome-host immune system crosstalk

The crosstalk between the microbiome and host immune system is fundamental to immune development and regulation, beginning as early as birth. The colonization of newborns by maternal and environmental microbes establishes the initial microbiome, which is critical for training the immune system. This early interaction influences the maturation of immune cells,

particularly regulatory T cells (Tregs) and innate immune components such as macrophages and dendritic cells. These interactions shape immune tolerance and protect against overactive inflammatory responses that could harm host tissues. [66-67]

Key signaling molecules produced by commensal bacteria facilitate this crosstalk. For instance, *Bacteroides fragilis* secretes polysaccharide A, which interacts with dendritic cells to promote Treg differentiation and suppress excessive inflammatory responses. Similarly, SCFAs like butyrate, acetate, and propionate, produced by gut microbes through the fermentation of dietary fiber, play a dual role. They enhance the integrity of the intestinal barrier while modulating systemic immune responses, reducing the risk of inflammation. Beyond the gut, microbiomes in other body sites contribute to localized immunity. The skin microbiome, for example, modulates keratinocyte activity and produces antimicrobial peptides, creating an environment unfavorable to pathogens.[68-69]

Microbiome-host crosstalk is not static; it evolves in response to diet, infections, antibiotics, and aging. Disruption of this crosstalk—whether by reduced microbial diversity or loss of beneficial species—can compromise immune functionality. The interconnectedness of the microbiome with systemic immunity emphasizes its role not just in local defense but also in broader immune regulation, linking microbial activity to conditions as diverse as infections, chronic inflammation, and even cancer.[70]

3.10.3 Dysbiosis: a link between microbiome imbalance and immune dysregulation

Dysbiosis, an imbalance in the composition or functionality of the microbiome, disrupts the finely tuned interaction between host and microbes, often triggering immune dysregulation. The causes of dysbiosis are multifactorial, including antibiotics, dietary changes, infections,

and environmental exposures. The resulting shifts in microbial populations can lead to reduced production of beneficial metabolites like SCFAs and increased colonization by opportunistic pathogens, altering immune responses at both local and systemic levels.[71]

One of the most studied effects of dysbiosis is the development of a "leaky gut," where increased intestinal permeability allows bacterial components such as lipopolysaccharides (LPS) to enter the bloodstream. This triggers systemic inflammation through the activation of toll-like receptor 4 (TLR4) on immune cells. Chronic exposure to these microbial products can drive the pathogenesis of immune-mediated conditions like IBD and RA. For instance, a decrease in *Faecalibacterium prausnitzii*, a key butyrate producer, is associated with heightened gut inflammation in IBD patients.[71-72]

Autoimmune diseases offer another striking example of dysbiosis-induced immune dysregulation. Alterations in the gut microbiome have been linked to conditions such as multiple sclerosis (MS) and type 1 diabetes (T1D). In MS, an overrepresentation of *Akkermansia muciniphila* and *Prevotella* species may exacerbate inflammation, while in T1D, reduced microbial diversity during early life is thought to impair immune tolerance. Dysbiosis also affects mucosal surfaces beyond the gut, such as the lungs, where changes in microbial populations have been implicated in asthma and chronic obstructive pulmonary disease (COPD).[73-74]

The implications of dysbiosis extend to therapeutic strategies. Restoring microbial balance through probiotics, prebiotics, dietary interventions, or fecal microbiota transplantation (FMT) has shown promise in alleviating immune-mediated conditions. For example, probiotics containing *Lactobacillus* and *Bifidobacterium* species are being explored for their ability to modulate immune responses and reduce inflammation in IBD and allergic diseases.

Understanding the specific mechanisms of dysbiosis and its impact on immune regulation is critical for developing these targeted interventions.[75-76]

The microbiome is an indispensable partner in immune regulation, with its intricate crosstalk ensuring a balance between immune tolerance and defense. Disruptions to this balance, as seen in dysbiosis, can have profound consequences for immune homeostasis, contributing to the development of chronic inflammatory diseases and autoimmunity. Continued research into the mechanisms of microbiome-host interactions will not only deepen our understanding of immune regulation but also provide the foundation for innovative therapeutic approaches aimed at restoring microbial and immune health.[75-76]

Chapter 4: Content and Results

4.1 THE ASSOCIATION BETWEEN MICROBIOTA DYSBIOSIS, INFLAMMATION AND IMIDS

IMIDs a diverse group of chronic conditions, are characterized by dysregulated immune responses targeting host tissues. Emerging research highlights the critical role of microbiota across various body sites—including the gut, skin, oral cavity, lungs, vagina, ear, eye, and urinary tract—in modulating immune homeostasis and contributing to the onset of IMIDs. Dysbiosis, defined as an imbalance in microbial composition, function, or distribution, has been increasingly implicated in the pathogenesis of these disorders.[77-79]

The gut microbiota is the largest and most diverse microbial community in the body, comprising trillions of microorganisms. It plays a pivotal role in digestion, immune system education, and the synthesis of bioactive compounds such as short-chain fatty acids (SCFAs). Dysbiosis in the gut, often driven by diet, antibiotics, infections, and genetic predispositions, disrupts the intricate host-microbe crosstalk, leading to increased intestinal permeability, systemic inflammation, and immune dysregulation. Conditions such as IBD, RA have been linked to reductions in anti-inflammatory taxa like *Faecalibacterium prausnitzii* and increases in pro-inflammatory species like *Enterobacteriaceae*. [78,79]

The skin microbiome serves as a frontline defense against pathogens and contributes to immune modulation. Dominated by species such as *Staphylococcus epidermidis* and *Cutibacterium acnes*, it maintains skin homeostasis by regulating immune responses and producing antimicrobial peptides. Disruptions in this ecosystem have been implicated in inflammatory

skin conditions such as atopic dermatitis, psoriasis, and acne vulgaris, wherein microbial imbalances promote localized inflammation. [80]

In the oral cavity, a complex microbial community—comprising genera such as *Streptococcus*, *Fusobacterium*, and *Actinomyces*—plays a crucial role in maintaining oral and systemic health. Dysbiosis in the oral microbiome is associated with periodontal disease, dental caries, and systemic conditions such as cardiovascular disease and rheumatoid arthritis, likely due to the translocation of inflammatory mediators and microbes into the bloodstream.[81]

The lung microbiome, once thought to be sterile, hosts a low-biomass community dominated by species such as *Prevotella*, *Veillonella*, and *Streptococcus*. It is essential for respiratory immune responses and maintaining lung health. Dysbiosis in the lung microbiota has been linked to chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis, where microbial imbalances exacerbate inflammation and impair lung function.[82]

The vaginal microbiome is critical for reproductive health, with *Lactobacillus* species dominating this ecosystem. These bacteria produce lactic acid, maintaining an acidic environment that inhibits pathogen colonization. Dysbiosis, characterized by a shift toward anaerobic bacteria, is associated with bacterial vaginosis, preterm birth, and increased susceptibility to sexually transmitted infections, underscoring its role in local and systemic immune regulation.[83]

The ear microbiome is a relatively small but significant microbial community that contributes to maintaining the health of the ear canal. Dominated by genera such as *Corynebacterium* and *Staphylococcus*, this microbiota protects against pathogenic colonization and supports the

immune defenses of the ear. Dysbiosis in the ear microbiome is linked to conditions such as otitis externa (swimmer's ear) and chronic otitis media, where microbial imbalances can drive localized inflammation.[52]

The eye microbiome, though low in biomass, plays an essential role in ocular health by modulating immune responses and preventing pathogenic infections. Key members include *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*. Dysbiosis in the eye microbiota has been implicated in conditions such as blepharitis, conjunctivitis, and keratitis. Alterations in microbial composition can disrupt the delicate balance needed for ocular surface immunity, leading to inflammation and susceptibility to infections. [84]

The urinary tract microbiome challenges the long-standing belief that the urinary tract is sterile. It is composed of diverse microorganisms, including species from the genera *Lactobacillus*, *Gardnerella*, and *Streptococcus*. This microbiota contributes to urinary health by preventing pathogen colonization through competitive inhibition and immune modulation. Dysbiosis in the urinary tract is associated with urinary tract infections (UTIs), interstitial cystitis, and overactive bladder syndrome, where microbial shifts may exacerbate inflammation and disrupt urinary function.[85]

Microbial metabolites, particularly SCFAs, are central to understanding the link between dysbiosis and inflammation. SCFAs influence immune function by modulating regulatory T cells (Tregs), enhancing epithelial barrier function, and suppressing inflammatory cytokine production. Dysbiosis-associated reductions in SCFA-producing bacteria across body sites have been correlated with disease severity, suggesting that therapeutic strategies aimed at restoring microbial balance could mitigate inflammation and disease progression.[68]

Moreover, increased permeability of epithelial barriers—whether in the gut, skin, eye—is a hallmark of many IMIDs. This permeability allows translocation of microbial products such as lipopolysaccharides (LPS), which activate toll-like receptors (TLRs) and downstream inflammatory pathways, perpetuating chronic inflammation. Bidirectional interactions between dysbiosis and immune dysregulation further compound disease processes, as immune responses can shape microbial composition and, conversely, microbial alterations can drive immune cell differentiation. [68]

These findings underscore the interconnectedness of microbiota across body sites and their systemic influence on health and disease. Approaches such as probiotics, prebiotics, dietary interventions, and fecal microbiota transplantation (FMT) hold promise in restoring microbial homeostasis and mitigating inflammation in IMIDs. However, the heterogeneity of IMIDs and interindividual variability in microbiota composition necessitate personalized strategies to maximize therapeutic efficacy. [68]

The interplay between microbiota dysbiosis, inflammation, and IMIDs is multifaceted and dynamic. A holistic understanding of host-microbe interactions across body sites is essential to developing innovative therapies that address the burden of IMIDs effectively.

4.1.1 Rheumatoid Arthritis and the Microbiome

Evidence points to significant alterations in the diversity of gut, oral, and dental microbiota between RA patients and healthy individuals. These microbial differences are closely associated with disease activity markers and autoantibody production, implying that the gut and oral microbiota may play a key role in initiating systemic inflammation, particularly in individuals with genetic predisposition. These alterations translate as a dysbiosis, or an

imbalance in the composition of gut microbiota, has been shown to disrupt local intestinal homeostasis, leading to chronic low-grade inflammation. This localized intestinal inflammation can subsequently evolve into systemic inflammation, contributing to the onset and progression of RA. Studies suggest that these microbial changes often occur before the clinical manifestation of RA, underscoring their potential as early biomarkers for disease prediction and prevention.[86,87]

Experimental data has supported the concept of antigen mimicry by microbial components, such as Sm and Fas proteins, which could activate immune responses via gut-associated lymphoid tissues (GALTs) and antigen-presenting cells (APC). Furthermore, the proliferation of RA-associated microbial species, like segmented filamentous bacteria (SFB) and certain *Lactobacillus* strains, disrupt microbial balance. This disruption leads to the release of pro-inflammatory molecules, including adenosine triphosphate (ATP), serum amyloid A, and chemokine ligand 5 (CCL5), which promote the activation of auto-reactive Th17 and Th1 cells. These immune cells can then migrate and trigger systemic autoimmune reactions. [87] Different sites of microbiome affect in different manners the RA pathology.

The oral cavity is a critical site for microbial interactions with the host immune system. Periodontitis, a common inflammatory disease of the gums, has been closely linked to RA. *Porphyromonas gingivalis*, a key periodontal pathogen, has been implicated in RA due to its ability to citrullinate host proteins via peptidylarginine deiminase (PAD). This citrullination process leads to the generation of anti-citrullinated protein antibodies (ACPAs), which are central to RA pathogenesis. Other oral bacteria, such as *Prevotella intermedia* and *Fusobacterium nucleatum*, have also been associated with systemic inflammation in RA. [88]

In RA, skin dysbiosis—characterized by a reduction in beneficial commensals and an increase in pathogenic species such as *Staphylococcus aureus*—may disrupt immune balance and exacerbate systemic inflammation. Emerging evidence links cutaneous microbial imbalances to heightened inflammatory responses, particularly through interactions with Toll-like receptors (TLRs) and the production of pro-inflammatory cytokines such as IL-1β and TNF-α. Dysbiosis on the skin may also enhance antigen presentation by Langerhans cells, amplifying autoimmune activity and contributing to RA disease progression. For example, recent studies have identified altered skin microbiota profiles in patients with systemic inflammatory diseases, suggesting a potential feedback loop between localized cutaneous inflammation and systemic immune activation. [88-91]

The biological pathways connecting gut dysbiosis to systemic inflammation have been extensively studied. The gut epithelial cells form a crucial dynamic barrier that regulates the passage of antigens through paracellular pathways. However, in the context of gut dysbiosis, this barrier's integrity can be compromised by the upregulation of zonulin, a protein responsible for modulating tight junctions between epithelial cells. Increased zonulin levels lead to the breakdown of tight junction proteins, a phenomenon observed in conditions such as rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, and other autoimmune diseases. When the gut barrier becomes permeable, it allows microbial components, such as lipopolysaccharides and other products, to translocate into the sub-epithelial space and lamina propria. These microbial fragments interact with APCs by binding to specific receptors, initiating an immune response. This activation drives the proliferation of pro-inflammatory T cell subsets, including T helper 1 (Th1) and T helper 17 (Th17) cells, which are known for their roles in producing inflammatory cytokines. Additionally, these interactions stimulate B cells to

differentiate into plasma cells capable of producing autoantibodies, further contributing to the systemic autoimmune response.[92]

The respiratory tract microbiota, particularly in the nasopharynx and lungs, is another potential contributor to RA. Studies suggest that microbial dysbiosis in the respiratory tract can lead to chronic inflammation and promote autoimmunity. *Prevotella* species, frequently detected in the lungs of RA patients, may induce Th17-mediated inflammation, which has been linked to both lung and joint pathology in RA.[88]

The genital and urinary microbiota, though less studied in the context of RA, may also play a role in modulating systemic immune responses. Alterations in microbial diversity in these niches could contribute to systemic inflammation through translocation of microbial components or direct immune activation. Emerging data indicate that urogenital infections or microbial imbalances may exacerbate systemic autoimmune conditions, including RA.[93,94]

The role of the microbiota in RA extends beyond the gut to include oral, gut, skin, respiratory, genital, urinary. A comprehensive understanding of the multi-niche microbiome in RA offers new opportunities for therapeutic interventions, including personalized microbiome modulation strategies aimed at restoring microbial balance and mitigating autoimmune responses. [87,88]

4.1.1.1 Evidence linking microbiota to RA

As stated in the previous chapters, RA is a chronic autoimmune disorder characterized by persistent inflammation of the joints, leading to cartilage destruction and bone erosion. Studies employing next-generation sequencing have revealed significant differences in gut microbiota profiles between individuals with RA and healthy controls. Notably, RA patients exhibit reduced abundances of beneficial taxa, such as *Faecalibacterium prausnitzii*, a known producer

of anti-inflammatory short-chain fatty acids (SCFAs). Concurrently, increases in potentially pathogenic species, such as *Prevotella copri*, have been documented. A landmark study demonstrated a higher prevalence of *P. copri* in the gut microbiota of early-onset RA patients, suggesting its potential involvement in the disease's initiation. Additionally, RA patients display a proliferation of the genus *Collinsella,* which has been linked to increased gut permeability and pro-inflammatory cytokine production. Some Lactobacillus species, often considered beneficial in other contexts, are also found in elevated levels in RA, potentially indicating a disease-specific role for these taxa. [86]

Cross-sectional studies further support these findings by linking microbial dysbiosis to disease severity and activity. RA patients with high disease activity often display heightened gut permeability—commonly referred to as "leaky gut"—which facilitates the translocation of microbial products such as lipopolysaccharides (LPS) into the systemic circulation. These microbial byproducts can activate immune pathways, exacerbating inflammation and joint damage.[88]

Longitudinal studies have begun to elucidate the temporal relationship between dysbiosis and RA development. For instance, alterations in gut microbiota composition have been detected in individuals with preclinical RA, characterized by the presence of autoantibodies such as anti-citrullinated protein antibodies (ACPAs), even before clinical symptoms arise. This suggests that dysbiosis may precede and potentially contribute to disease onset. [88-90]

4.1.1.2 Mechanisms: microbial triggers of autoimmunity in RA

The gut microbiota can influence immune responses through multiple mechanisms, some of which may drive autoimmune processes in RA. But we can gather them under 4 primary

mechanisms: post-translational modifications, inflammatory responses, molecular mimicry, loss of intestinal barrier integrity (Figure 4.1) The modification of self-proteins through post-translational processes, particularly citrullination, is a critical factor in the pathogenesis of rheumatoid arthritis (RA). Citrullination involves converting arginine residues into citrulline on peptide chains. This process, mediated by peptidylarginine deiminases (PADs), occurs during normal cellular functions such as differentiation, apoptosis, and the formation of neutrophil extracellular traps (NETs). However, under specific circumstances, excessive citrullination—referred to as hypercitrullination—can arise. This overactivity creates novel epitopes that evade immune tolerance, leading to the production of anti-citrullinated protein antibodies (ACPAs), which are detectable in the early, preclinical stages of RA. Hypercitrullination is often linked to the overactivation of PAD enzymes triggered by cell damage, necrosis, or NETosis. Environmental factors, including smoking and exposure to certain bacteria at mucosal sites, further amplify these processes. [86-90]

Two bacteria found in the oral cavity, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans,* have been implicated in driving citrullination. *P. gingivalis* produces a specialized enzyme, Porphyromonas PAD (PPAD), which can citrullinate both bacterial and host proteins, such as fibrinogen and α-enolase—common ACPA targets. This bacterial citrullination may provoke immune responses that cross-react with self-peptides, potentially leading to a breakdown of immune tolerance and the production of ACPAs. However, a key difference exists between human PADs and PPAD; the former citrullinate internal arginine residues, whereas PPAD primarily modifies C-terminal arginines, leaving the precise mechanism under debate. [86-90]

Aggregatibacter actinomycetemcomitans contributes to hypercitrullination through its toxin leucotoxin A (LtxA), which creates pores in neutrophil membranes, triggering calcium-dependent activation of PADs. This results in increased generation of citrullinated antigens, further driving ACPA formation. Both bacteria are strongly associated with periodontal disease, which may partially explain the well-documented epidemiological link between RA and periodontitis. [86-90]

Secondly the molecular mimicry, whereby microbial antigens share structural similarity with host proteins, leading to cross-reactive immune responses. Certain microbial antigens share structural similarities with human proteins, leading to the generation of cross-reactive autoantibodies. For example, *Prevotella copri* has been implicated in producing antigens that mimic human proteins, potentially triggering the production of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs), key markers of RA. [86-90]

Another key mechanism is the inflammatory responses via modulation of T cell subsets by gut microbiota. Dysbiosis can skew the balance between pro-inflammatory and regulatory immune responses, favoring the expansion of Th17 cells while reducing regulatory T cells (Tregs). Th17 cells secrete interleukin-17 (IL-17) along with TNF-α, cytokines that promote synovial inflammation, joint damage and bone resorption, thereby contributing to RA pathogenesis. Conversely, a decrease in Tregs compromises immune tolerance and allows for sustained inflammation. [87-91]

The production of SCFAs, particularly butyrate, by gut bacteria is another critical factor. SCFAs enhance epithelial barrier integrity, regulate T cell differentiation, and suppress inflammatory cytokines. Reduced SCFA production due to the loss of beneficial microbes like *F. prausnitzii* can impair these protective mechanisms, exacerbating systemic inflammation in RA.[92]

In addition to immune modulation, gut dysbiosis can impact the metabolism of tryptophan, an essential amino acid with immunoregulatory functions. Altered microbial metabolism in RA patients leads to increased production of metabolites that activate the aryl hydrocarbon receptor (AhR). This receptor influences immune responses and may contribute to the inflammatory milieu observed in RA. [87]

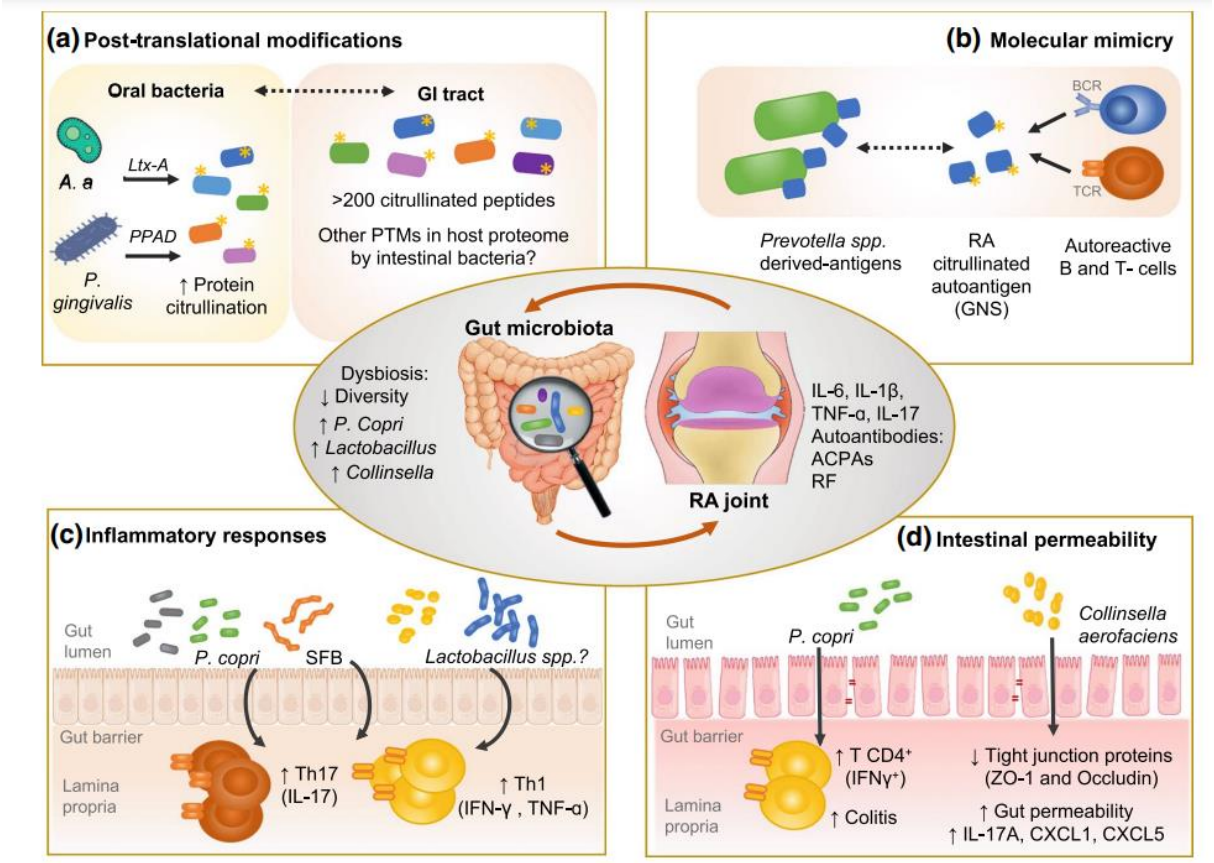

Figure 4.1 Molecular mechanisms with altered-gut microbiota in rheumatoid arthritis (RA) [89]

Leucotoxin-A (Ltx-A), Porphyromonas-PAD (PPAD), N-acetylglucosamine-6-sulfatase (GNS), tight junction proteins (ZO-1 and occludin), segmented flamentous bacteria (SFB)

Furthermore, microbial-derived products such as LPS and peptidoglycans can breach the gut barrier during dysbiosis and interact with toll-like receptors (TLRs) on immune cells. These interactions cause the loss of intestinal barrier integrity and activate downstream inflammatory signaling pathways, perpetuating chronic inflammation characteristic of RA. In other words this allows microbial-derived components, such as LPS, to translocate into the bloodstream, triggering systemic immune activation. The subsequent release of pro-inflammatory cytokines exacerbates synovial inflammation and promotes joint destruction. In a mouse model of autoimmune-induced arthritis (AIC), administration of *Collinsella aerofaciens*—a bacterium found in elevated levels in some rheumatoid arthritis (RA) patients—was shown to increase both the incidence and severity of arthritis. This bacterium disrupted intestinal epithelial integrity by downregulating the expression of tight junction proteins, such as ZO-1 and occludin, in the human colorectal adenocarcinoma epithelial cell line (CACO-2). Simultaneously, it increased the expression of pro-inflammatory mediators, including interleukin-17A (IL-17A), chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand 5 (CXCL5), and nuclear factor kappa B1 (NF-κB1). [86-94]

Similarly, oral exposure of C57BL/6 mice to *Prevotella copri* led to an increased population of interferon-gamma-producing CD4+ T lymphocytes (IFN-γ+ CD4+ T cells) and severe colitis after treatment with dextran sulfate sodium (DSS). Interestingly, individuals at risk for RA were observed to have fewer IFN-γ-producing group 1 innate lymphoid cells (ILC1s), while patients in the early stages of RA demonstrated a rise in interleukin-17-producing group 3 innate lymphoid cells (ILC3s). These findings suggest that immune cell profile changes occur within the gut during the early phases of the disease. [86-94]

These observations underscore the role of intestinal bacteria in driving inflammation and impairing gut barrier integrity. By influencing the GALT, these microbes may weaken the intestinal barrier, enabling bacterial antigens to enter systemic circulation and potentially initiate inflammatory immune responses at sites beyond the intestine. [86-92]

Collectively, these mechanisms highlight the multifaceted role of gut microbiota in RA, providing valuable insights into potential therapeutic strategies. Interventions aimed at restoring microbial balance, such as probiotics, prebiotics, and dietary modifications, are emerging as promising approaches to mitigate inflammation and slow disease progression. However, further research is needed to fully elucidate causal relationships and develop targeted microbiome-based therapies for RA. [89-94]

4.1.2 Ankylosing Spondylitis and the Microbiome

As it is mentioned in the previous chapters; AS is a chronic, inflammatory disease primarily affecting the axial skeleton, leading to pain and progressive spinal fusion. The disease is also defined by radiographic axial spondyloarthritis (axSpA). The exact mechanisms underlying the development of ankylosing spondylitis (AS) remain incompletely understood. However, the presence of the HLA-B27 gene, found in approximately 90% of individuals diagnosed with AS, is recognized as a significant risk factor for the condition. A recent hypothesis suggests a connection between the HLA-B27 allele and the composition of the intestinal microbiota, referred to as the "B-27 shaped flora," in contributing to the increased risk of AS. Supporting evidence includes studies on HLA-B27 transgenic rats raised in germ-free environments, which do not exhibit an arthritic phenotype. However, when these rats are recolonized with commensal microbiota, over 80% develop both arthritis and colitis, demonstrating a link between HLA-B27–microbiota interactions and disease susceptibility. Additionally, the HLA-

B27 allele has been shown to significantly alter the intestinal microbiome of healthy individuals without AS, suggesting that HLA-B27-associated dysbiosis may precede clinical symptoms and contribute to disease initiation. Despite these findings, current evidence remains circumstantial, and further research is needed to clarify the exact role of HLA-B27 in promoting gut dysbiosis and the subsequent development of AS. It is also important to state that around 30% of patients with inflammatory bowel disease (IBD) develop spondyloarthritis (SpA), while 10–15% of individuals with SpA have a history of IBD. Furthermore, approximately 60% of SpA patients without overt gastrointestinal symptoms show evidence of mild intestinal inflammation. Although the mechanisms linking intestinal inflammation to SpA onset remain uncertain, gut microbiota appears to play a pivotal role in the gut-joint axis.[95,96]

4.1.2.1 Evidence Linking Gut Microbiota to AS

Research has shown that HLA-B27 transgenic rats raised in germ-free environments do not develop SpA or arthritis; however, introducing gut microbiota triggers both arthritis and intestinal inflammation. Similar findings have been reported in human studies, where the gut microbiota of HLA-B27-positive individuals differs significantly from that of healthy controls, suggesting its critical role in SpA development. One hypothesis is that HLA-B27 presents specific microbial peptides to CD8+ T cells, eliciting a pathogenic immune response. The gut microbiota produces a diverse array of microbial peptides, which may activate these T cells. Supporting this theory, increased populations of CD8+ T cells have been observed in the peripheral blood and synovium of patients with SpA and AS. Interestingly, the microbiota's role appears to vary across animal models. For instance, *Prevotella, Clostridium,* and *Brucella* are implicated in SpA development in Lewis rats, while *Akkermansia* and *Lactobacillus* are more influential in Fischer rats.[8,96]

A study by Uchiyama and colleagues reported distinct microbial patterns in SpA patients, including higher relative abundances of *Lachnospiraceae*, *Ruminococcaceae*, *Rikenellaceae*, *Porphyromonadaceae*, and *Bacteroidaceae*, coupled with lower levels of *Veillonellaceae* and *Prevotellaceae*. Unlike rheumatoid arthritis (RA), where specific microorganisms are thought to play key roles, the progression of SpA may involve the interplay of multiple microbial species, mediating inflammation and activating effector cells. [96]

Klebsiella pneumoniae is the first bacterium linked to AS and is believed to precede HLA-B27-associated changes. While usually a commensal organism, it can act as an opportunistic pathogen under certain conditions. In a 1976 study, *K. pneumoniae* was detected in 76% of AS patients' fecal samples compared to less than 30% in healthy controls. Antisera prepared against *K. pneumoniae* isolates demonstrated cytotoxic effects on lymphocytes from HLA-B27-positive AS patients but not from HLA-B27-negative controls, indicating its potential role in AS pathogenesis. Furthermore, the molecular mimicry hypothesis suggests that *K. pneumoniae*'s nitrogenase reductase shares six consecutive amino acids with HLA-B27, potentially triggering autoimmune responses. Despite these findings, the role of *K. pneumoniae* remains controversial, as much of the evidence stems from serological studies. [96]

Recent research has also explored broader microbial changes in AS. For example, 16S rRNA sequencing of fecal samples revealed lower abundances of *Bacteroides* and higher levels of *Firmicutes* and *Verrucomicrobia* in AS patients. These changes correlated with increased C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), markers of systemic inflammation. Metagenomic studies identified elevated levels of *Bacteroides coprophilus*, *Parabacteroides distasonis*, *Eubacterium siraeum*, *Acidaminococcus fermentans*, and

Prevotella copri in AS patients, along with enhanced pathways for oxidative phosphorylation, lipopolysaccharide biosynthesis, and glycosaminoglycan degradation. [96]

In a study by Francesco et al., ileal biopsies were performed on a group of 50 patients with HLA-B27-positive AS and 20 healthy controls. The analysis identified invasive and adherent bacteria, primarily gram-negative strains such as *Escherichia coli* and *Prevotella*, in the ileum of AS patients. These bacterial populations were linked to a decrease in tight junction (TJ) proteins and disruption of the gut vascular barrier (GVB), suggesting a role in intestinal and systemic inflammation. [8]

Collectively, these findings suggest that in AS, no single microbial species plays a dominant role. Instead, the disease may result from complex interactions among multiple microbial communities. However, as microbial research continues to evolve, identifying key bacterial players and their contributions to AS pathogenesis may pave the way for microbiota-targeted therapies.

4.1.2.2 Microbial Triggers of Inflammation in AS

The gut microbiota contributes to inflammation in AS through several mechanisms. One prominent hypothesis is molecular mimicry, where microbial antigens resemble self-antigens, leading to an autoimmune response. For instance, *Klebsiella pneumoniae* shares peptide sequences with HLA-B27, a genetic marker strongly associated with AS. This cross-reactivity may drive the inflammatory processes characteristic of AS. *Klebsiella*, in particular, has been implicated in molecular mimicry, where bacterial antigens may trigger immune responses against host tissues. [8,96-98]

Another proposed mechanism involves the misfolding of HLA-B27 within the endoplasmic reticulum, causing stress that triggers responses such as autophagy or the unfolded protein response. Microorganisms also play a crucial role in AS development, as molecular mimicry can promote autoimmunity and immunological cross-reactivity. Research has shown that disease severity in AS patients is associated with alterations in gut microbiota, with species such as *Ruminococcus gnavus*, *Erysipelatoclostridium ramosum*, *Clostridium symbiosum*, and *Clostridium bolteae* being more abundant in those with severe forms of the disease.[97]

The link between the gut microbiome and joint inflammation may arise from metabolites it produces, including short-chain fatty acids, tryptophan derivatives, amino acids, trimethylamine, and B vitamins. These compounds regulate gut barrier integrity, T-cell activity, and cytokine production. For example, tryptophan-derived metabolites like indole-3-acetate and indole-3-acetaldehyde are elevated in axial spondyloarthritis regardless of intestinal inflammation, unlike bacterial indole produced by tryptophanase.[96-98]

Other pathogens beyond gut microbiota are also implicated in AS development. *Candida* infections have been associated with an increased risk of AS several years after infection. Similarly, human papillomavirus (HPV) infection significantly raises the risk of AS and has the potential to trigger systemic lupus erythematosus. Among individuals with human immunodeficiency virus (HIV), AS shows a strong association compared to other autoimmune conditions.[97-99]

As evidence linking the gut microbiome to AS continues to grow, advancements in microbiome research have opened avenues for personalized diagnostic and therapeutic approaches. Tailored treatments, including dietary supplements targeting specific microbial populations, hold promise for managing AS more effectively.[8,96,97]

Furthermore, increased gut permeability observed in AS patients may facilitate the translocation of microbial components into systemic circulation, driving systemic inflammation. (Fig 4.2) [100]

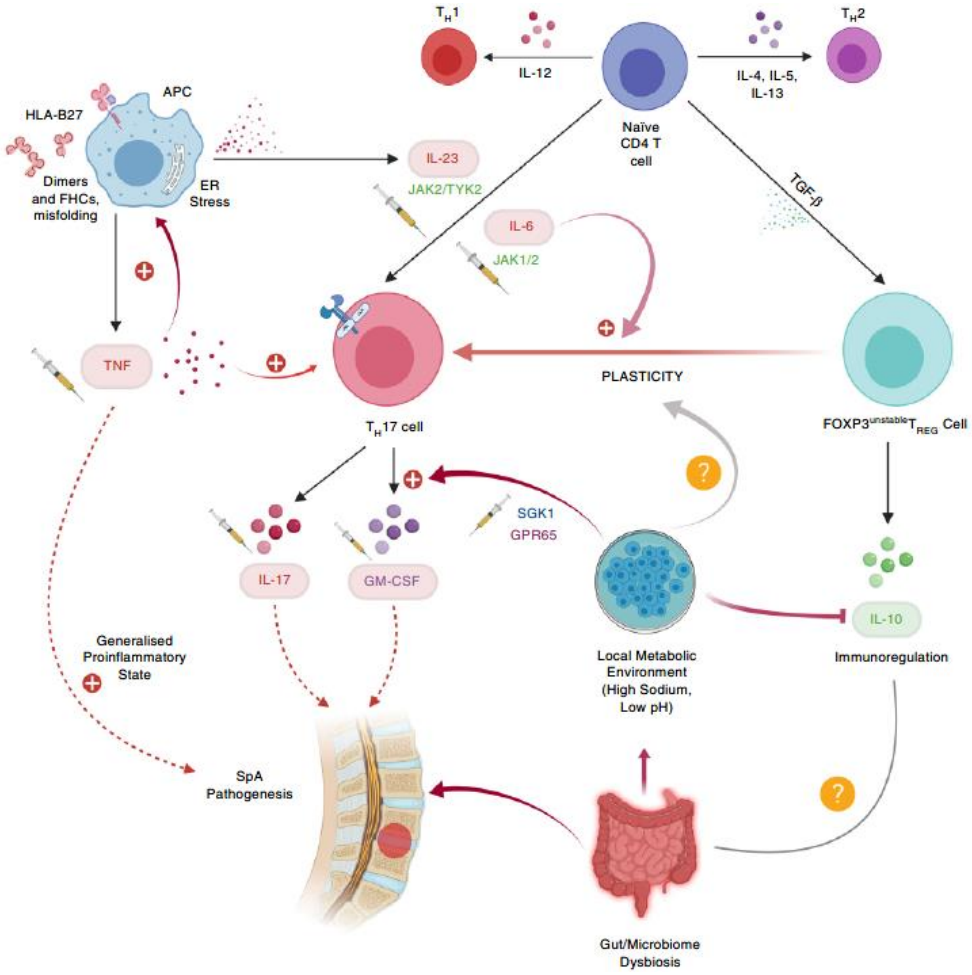

Figure 4.2 Factors affecting CD4+ T-cell differentiation and their roles in AS pathogenesis. Current or future drug targets are indicated with a syringe symbol [100]

Cluster of differentiation 4 (CDK 4+), Januse Kinase (JAK), Serum and glucocorticoid-regulated kinase 1(SGK 1), Tyrosine Kinase 2 (TYK-2)

Additionally, microbial dysbiosis can promote an imbalance in T cell subsets, particularly increasing Th17 cells, which are potent producers of the pro-inflammatory cytokine interleukin-17 (IL-17). Since the IL23/IL17 axis plays a key role in the pathogenesis and elevated IL-17 levels in AS patients are thought to contribute to bone erosion and inflammation. Altered gut microbiota also affects the production of metabolites such as short-chain fatty acids (SCFAs), which normally regulate immune responses. A decrease in SCFAs may compromise intestinal barrier integrity, further exacerbating inflammation.[96-100]

Finally, after the discovery of these mechanisms, some therapeutic strategies were implied in order to achieve treatment goals. These strategies is elaborated under the section title "microbiome based therapies". [101]

4.1.3 Psoriatic Arthritis and the Microbiome

PsA is a type of inflammatory arthritis that occurs in association with psoriasis, presenting with symptoms such as joint pain, swelling, and characteristic skin lesions. Emerging research highlights the significant roles played by both the gut and skin microbiomes in the pathogenesis of PsA. Many individuals with PsA also experience subclinical gut inflammation, which may contribute to disease development.[102]

Studies have shown a reduction in gut microbial diversity among PsA patients, with specific taxa being notably affected. For instance, *Coprococcus* has been inversely associated with both psoriasis and PsA, whereas reduced levels of *Ruminococcus* and *Akkermansia* are unique to PsA. Interestingly, the diminished abundance of *Ruminococcus* in PsA mirrors findings in individuals with IBD, highlighting a potential overlap in microbiome alterations between these

conditions. In contrast, the reduction of *Akkermansia* in PsA differs from its patterns in juvenile spondyloarthritis (SpA), suggesting that distinct microbial compositions may influence the development of different inflammatory diseases.[102,103]

In addition to *Ruminococcus* and *Akkermansia*, other short-chain fatty acid (SCFA)-producing bacteria, including *Faecalibacterium* and *Prevotella*, are found to be less abundant in conditions such as psoriasis, PsA, IBD, and obesity (Figure 4.3). These bacteria play a critical role in gut homeostasis by producing SCFAs, which have anti-inflammatory properties and help maintain the integrity of the gut barrier. Furthermore, *Alistipes*, a commensal organism commonly found in healthy individuals, is significantly reduced in both PsA and Crohn's disease.[103]

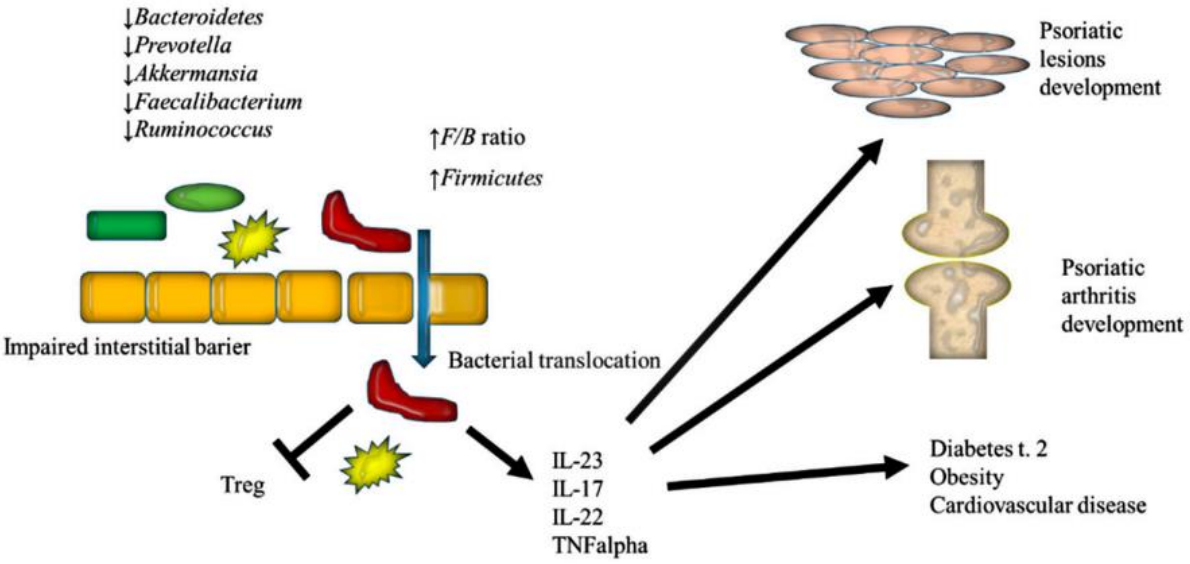

Figure 4.3 The impact of gut dysbiosis in psoriasis on the development of psoriatic lesions, PsA, and psoriasis comorbidities (F/B: Firmicutes to Bacteroidetes ratio) [103]

A hallmark of dysbiosis in PsA appears to be the loss of beneficial commensals, which can disrupt the delicate balance of the immune system. This microbial imbalance may contribute to chronic inflammation and disease progression, underscoring the need for further investigation into the gut-skin axis and its role in PsA pathogenesis.[102,103]

4.1.3.1 Skin and Gut Microbiome Alterations in PsA

Previous studies highlighted the role of gut microbiota in driving arthritis and colitis development in HLA-B27 transgenic rats. Since then, a growing body of evidence has reinforced the hypothesis that gut microbial dysbiosis can trigger an exaggerated immune response in genetically predisposed individuals. This overactivation in the intestinal lamina propria can escalate into systemic inflammation, eventually manifesting as joint disease. The relevance of HLA-B27 and associated genes has been substantiated in conditions such as ankylosing spondylitis, inflammatory bowel disease, and psoriatic arthritis. Histological and molecular markers of subclinical gut inflammation have been detected in patients with AS and PsA, further supporting this connection. Novel findings revealed a reduction in gut microbiota diversity in patients with PsA and psoriasis. This decreased diversity was largely attributed to a reduced abundance of specific bacterial taxa. Certain reductions, such as those in *Coprococcus* (OTU 43), were observed in both conditions, indicating a shared gut microbiome signature in skin psoriasis and PsA. However, other taxa showed condition-specific patterns, with *Ruminococcus* and *Akkermansia* uniquely diminished in PsA. The reduced microbial diversity observed in PsA and IBD is primarily driven by a loss of phylogenetically related taxa. For example, *Akkermansia*, *Ruminococcus*, and *Alistipes* are significantly underrepresented in IBD and PsA patients. *Akkermansia muciniphila*, a key mucin-degrading symbiont found in

healthy individuals, converts mucin into SCFAs like acetate and propionate, which support epithelial activation and proper immune regulation. Its absence in PsA cohorts suggests a diminished protective function. Similarly, *Ruminococcus species*, excluding *R. gnavus*, are known for their role in SCFA production and gut homeostasis and are also depleted in Crohn's disease. *Alistipes*, another health-associated genus, is markedly reduced in patients with ileal Crohn's disease, making it another shared taxon between IBD and PsA. Additionally, reductions in *Coprococcus* (OTU 43) in PsA patients were linked to lower levels of medium-chain fatty acids (MCFAs) like hexanoate and heptanoate. MCFAs are known for their antibacterial properties and their ability to activate PPAR-γ, a key regulator of intestinal inflammation. The potential role of MCFA-induced PPAR-γ activation in PsA warrants further investigation.[103-104]

Collectively, the loss of these microbial taxa may impair the host's ability to regulate intestinal immune responses. This disruption could lead to broader inflammation, manifesting as gut-related disorders such as Crohn's disease or extra-intestinal conditions like PsA. Elevated concentrations of secretory IgA (sIgA) observed in PsA patients suggest a localized mucosal immune reaction, potentially indicative of efforts to limit inflammation within the gut. Alternatively, this could signal a compromised gut barrier, possibly linked to the early stages of PsA.[104]

Interestingly, fecal levels of RANKL were selectively low in PsA patients compared to psoriasis and healthy controls. This finding is significant given the role of RANKL in systemic PsA pathogenesis, where it is elevated in serum, synovium, and psoriatic skin. RANKL's intestinal function includes the differentiation of microfold (M) cells, which are essential for antigen sampling. A reduction in fecal RANKL might reflect shifts in gut microbial composition or a

broader imbalance in the RANKL/OPG axis, with implications for inflammation dissemination to joints.[104]

Previously, studies in treatment-naïve rheumatoid arthritis patients identified an expansion of *Prevotella copri* as a potential trigger in susceptible individuals. However, in PsA patients, no such expansion was observed, underscoring distinct microbial patterns between these conditions. These differences highlight the complexity of the gut microbiome's role in autoimmune diseases and emphasize the need for further mechanistic studies to elucidate its contribution to PsA and other SpA pathologies.[104]

4.1.3.2 Mechanisms of Dysbiosis in PsA and its effect

Gut dysbiosis has been linked to genomic research findings, which demonstrate that polymorphisms in signaling pathways, particularly those involving interleukin-23 (IL-23), are associated with the development of inflammatory disorders, including PsA and IBD. Key insights have emerged from studies using mouse models, such as the SKG model of arthritis and enteritis, which involves a ZAP70 mutation. This mutation disrupts signaling pathways mediated by T-cell antigen receptors (TCR) and the IL-23/IL-17 axis. In these models, exposure to microbial components such as β1,3-glucan induced enteritis and arthritis. However, disease development was prevented when the mice were maintained under germ-free conditions. [103]

Similarly, TNF-overexpressing mice (TNFΔARE/+), commonly utilized as models for SpA and IBD, did not develop ileitis when raised in microbial-free environments. Recent findings have further demonstrated that germ-free TNFΔARE/+ mice fail to exhibit Crohn-like ileitis, highlighting the critical role of microbial exposure in disease manifestation.[103]

The regulation of intestinal microbial balance is facilitated by a variety of immune cells. Among these, regulatory T cells (Tregs), characterized by the markers FOXP3, CD25, and CD4, play a vital role in maintaining immune tolerance. Innate-like T cells, including γδ T cells, invariant natural killer T cells (iNKT), and mucosal-associated invariant T cells (MAIT), also contribute significantly to immune modulation. These cells can secrete a wide range of cytokines, such as IL-17, TNF, IL-22, IL-10, IFN-γ, and IL-4, through both TCR-dependent and independent mechanisms. [103]

iNKT cells, which recognize bacterial glycolipids through CD1d receptors, and MAIT cells, which respond to bacterial or yeast-derived riboflavin metabolites, can exhibit both pro-inflammatory and anti-inflammatory roles depending on the context. Their phenotypes may also be influenced by substances produced by intestinal bacteria or through interactions with Tregs. [103]

Various hypotheses connect the development of spondyloarthropathies to disruptions in gut microbiota. One such hypothesis suggests that intestinal dysbiosis arises during childhood due to factors such as delivery method, early antibiotic use, or breastfeeding practices. This dysbiosis is thought to impair the normal development and adaptation of the innate immune system, potentially leading to chronic inflammation. Supporting this theory, research has shown that mice lacking secondary lymphoid organs due to the absence of lymphotoxin exhibit specific gut microbiota imbalances. These imbalances promote IL-17 production and the onset of autoimmune diseases. [103]

Another hypothesis emphasizes a dynamic relationship between genetic predispositions, the immune system, and gut microbiota. Studies have shown that antibiotic treatment in adult mice reduces the severity of imiquimod-induced psoriasis-like dermatitis. However, administering

the same antibiotics early in life exacerbates inflammation during adulthood, evidenced by elevated IL-22-producing γδ T cells in response to imiquimod or recombinant IL-23 exposure. These findings underscore the critical role of gut microbes in autoimmune disease progression. [103]

Further evidence suggests a link between breastfeeding practices and autoimmune conditions; for example, individuals with ankylosing spondylitis were breastfed less frequently during infancy compared to the general population. Additionally, studies in rats have revealed that HLA-B27 expression significantly alters the intestinal metabolome, further emphasizing the interplay between genetic factors and microbial environments. [103-104]

4.1.4 Psoriasis and the Microbiome

Psoriasis, a chronic inflammatory skin condition, has been extensively associated with microbial dysbiosis. The gut microbiota in psoriasis patients often exhibits reduced microbial diversity, with a decrease in SCFA-producing bacteria and an increase in inflammatory species like Proteobacteria. These changes may disrupt immune homeostasis, contributing to systemic inflammation observed in psoriasis.[103]

4.1.4.1 Microbial Triggers in Psoriasis

The skin microbiome acts as a protective barrier against pathogens and regulates immune responses. In healthy individuals, it is dominated by commensal species such as *Cutibacterium acnes* and *Staphylococcus epidermidis*. In psoriasis, however, an overrepresentation of pathogenic species such as *Staphylococcus aureus* and *Malassezia* has been observed. These changes contribute to skin inflammation and exacerbate psoriasis symptoms. [103]

Psoriatic lesions are associated with notable disruptions in the skin microbiota. Studies have reported a decline in the populations of *Corynebacterium spp*., *Lactobacillus spp*., *Burkholderia spp*., and *Cutibacterium acnes* in psoriatic skin compared to healthy controls. Non-culture-based microbiome analysis also highlights reduced levels of *Burkholderia*, *Corynebacterium*, *Lactobacillus*, and *Cutibacterium*, along with an increased prevalence of *Streptococcus*. Interestingly, the *Firmicutes* phylum is overrepresented in psoriatic skin, whereas the *Actinobacteria* phylum is diminished. Research has shown that *Corynebacterium* abundance positively correlates with Psoriasis Area Severity Index (PASI) scores, suggesting its role in disease severity. Furthermore, this genus may influence interferon signaling pathways, potentially contributing to skin dysbiosis and the formation of psoriatic lesions. Scratching caused by itching in psoriasis often compromises the skin barrier, allowing bacteria, including epidermal colonizers, to penetrate the dermis or peripheral blood. This direct interaction with immune cells triggers innate and adaptive inflammatory responses, further exacerbating skin dysbiosis.[102,103]

Fungal diversity is notably higher in psoriatic lesions than in healthy skin, with *Malassezia* being the most abundant genus. While the role of *Malassezia* in psoriasis pathogenesis remains unclear, *Malassezia sympodialis* has been shown to increase keratinocyte proliferation and stimulate pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-8. Other studies suggest that this fungus may promote the pro-inflammatory maturation of dendritic cells and enhance mast cell activity, both of which contribute to inflammation in psoriatic lesions.[103]

The gut microbiome in psoriasis shares similarities with that observed in IBD. Both conditions exhibit a reduction in beneficial microbes, including *Faecalibacterium prausnitzii*, *Bifidobacterium* spp., *Lactobacillus* spp., *Parabacteroides*, and *Coprobacillus*. In contrast,

opportunistic pathogens such as *Salmonella*, *Campylobacter*, *Helicobacter*, *Escherichia coli*, *Alcaligenes*, and *Mycobacterium* are often more abundant.[103]

In psoriasis, an elevated Firmicutes-to-Bacteroidetes (F/B) ratio is commonly observed, reflecting an impaired gut epithelial barrier and systemic inflammation. This microbial imbalance reduces the production of butyrate, a short-chain fatty acid vital for maintaining intestinal homeostasis and modulating inflammatory responses. Reduced butyrate levels have been linked to insulin resistance and increased production of acetate, a precursor of appetite-regulating hormones.[103]

Reduced abundances of *Ruminococcus* and *Akkermansia* have also been noted in the gut microbiota of psoriasis and psoriatic arthritis patients. Conversely, there is an increase in *Lachnospiraceae*, *Ruminococcaceae*, *Collinsella aerofaciens*, and *Dorea formicigenerans*. The underrepresentation of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, both crucial for intestinal health, underscores the extent of gut dysbiosis in psoriasis.[103]

Gut dysbiosis in psoriasis impacts immune responses at both intestinal and systemic levels. Elevated levels of IL-1α, a key cytokine in inflammation, have been detected in the intestinal lumen of psoriasis patients. This cytokine is involved in antigen presentation, Th17 cell activation, and links intestinal inflammation to skin lesions.[103]

4.1.4.2 Mechanisms of Skin-Gut Dysbiosis in Psoriasis

The interplay between skin and gut microbiomes, often referred to as the "gut-skin axis," highlights their collective role in disease progression. The interaction between commensal microorganisms and the host is mediated through the recognition of microbial-associated

molecular patterns (MAMPs) by pattern recognition receptors (PRRs). This interaction enables the microbiota to influence and shape the development of the human immune system after birth.

Research highlights a continuous interplay between Toll-like receptors (TLRs), peptidoglycan-recognition proteins, antimicrobial peptides, cytokines, and the skin microbiota. For instance, keratinocytes produce cathelicidins (LL-37), a type of antimicrobial peptide, upon contact with commensal microbes. These peptides bind to nucleic acids released from epithelial cells undergoing apoptosis, often triggered by external factors like bacterial infections, viral exposure, or mechanical damage in genetically predisposed individuals. The interaction between LL-37 and self-DNA activates plasmacytoid dendritic cells (pDCs), driving the production of type I interferons. Similarly, LL-37 binding to self-RNA stimulates myeloid dendritic cells (mDCs) to release TNF-α and inducible nitric oxide synthase (iNOS). These cytokines, in turn, promote the differentiation of naive T cells into Th17 cells, which produce interleukins IL-17 and IL-22, both implicated in the formation of psoriasis lesions. [103]

The role of *Candida albicans* in psoriasis pathogenesis has also been explored. Dendritic cells exposed to fungal ligands such as β-glucan induce the secretion of IL-36α, which contributes to the development of a psoriasiform phenotype. Furthermore, animal models of psoriasis have shown that mice treated with antibiotics or raised in germ-free conditions exhibit reduced skin inflammation and fail to develop psoriasis-like plaques. These findings underscore the potential contribution of the microbiome to skin inflammation and the emergence of psoriasis.[103,104]

Another noteworthy mechanism involves the M protein of *Streptococcus pyogenes*, a bacterium that frequently colonizes psoriatic skin. This protein exhibits molecular mimicry with the 50-kDa type I keratin, leading to the activation of autoreactive T cells and triggering inflammatory processes that contribute to psoriasis.[103,104]

The critical role of the skin microbiome in psoriasis is further supported by the correlation between beta defensin, an antimicrobial protein found in the blood and skin, and IL-17 levels, a key inflammatory cytokine in psoriasis pathogenesis. Beta defensin levels have been observed to decrease following treatment with secukinumab, an anti-IL-17 antibody, and this reduction is directly linked to improvements in the Psoriasis Area and Severity Index. Further research is needed to determine whether the observed alterations in the skin microbiome are a cause or a consequence of successful psoriasis treatments.[103]

According to the association between the gut dysbiosis and its effect on psoriasis, recent studies demonstrated that inflammatory skin conditions in murine models, specifically keratinocyte-specific caspase-1 transgenic mice, were associated with an abundance of *Staphylococcus aureus* and *Streptococcus danieliae* in the gut. Administering these bacteria exacerbated skin lesions and elevated levels of pro-inflammatory cytokines such as IL-17A, IL-17F, IL-22, and TNF-α. Similarly, another research highlighted compromised intestinal barrier integrity in psoriasis patients, as evidenced by elevated serum levels of intestinal fatty acid-binding protein (I-FABP) and claudin-3. These findings underscore the importance of intestinal dysbiosis in the pathogenesis of psoriasis. [103-106]

This interplay may create a feedback loop, where skin inflammation fosters specific gut microbial imbalances that, in turn, aggravate the skin condition. Recent studies revealed that bacterial translocation in psoriasis patients leads to the presence of bacterial DNA in the bloodstream, correlating with earlier disease onset, prolonged disease duration, and heightened levels of pro-inflammatory cytokines.[103]

Certain gut bacteria, including species known for promoting regulatory T cells (Tregs), exhibit anti-inflammatory effects and help maintain immune tolerance. These species also inhibit Th17

cells, a key driver in psoriasis pathogenesis. Eppinga et al. have highlighted the potential systemic and immunomodulatory roles of *Faecalibacterium* and *Akkermansia* when these bacteria are deficient. Furthermore, *Bifidobacterium* has demonstrated anti-inflammatory properties in colitis models, enhancing Treg production. Clinical studies with psoriasis patients indicate that supplementation with *Bifidobacterium* reduces serum levels of C-reactive protein (CRP) and TNF-α. [103]

In psoriasis and psoriatic arthritis, changes in both the intestinal microbiota and immune response components have been documented. Patients' fecal samples reveal increased levels of soluble IgA and decreased levels of receptor activator of nuclear factor kappa-B ligand (RANKL). While RANKL is overexpressed in the serum and joints of psoriatic arthritis patients, it plays a distinct role in intestinal immune cell differentiation. Variations in its expression may reflect the impact of microbiota or indicate its broader role in systemic inflammation typical of psoriasis. [103-105]

Elevated IL-1α levels have also been observed in the intestines of psoriasis patients. This cytokine, a critical player in inflammation, promotes T-cell accumulation, antigen presentation, and Th17 activation. Its increased intestinal expression could link gut inflammation with psoriatic skin lesions.[103-105]

The association between gut dysbiosis in psoriasis and related comorbidities is evident (Figure 4.4). Elevated Firmicutes-to-Bacteroidetes ratio is also observed in conditions like cardiovascular disease, type 2 diabetes, and obesity. Studies show that this imbalance correlates with increased levels of trimethylamine-N-oxide (TMAO), a metabolite that promotes atherogenesis and systemic inflammation. Reduced short-chain fatty acid production due to

dysbiosis further contributes to inflammation, insulin resistance, and metabolic disorders. [103-107]

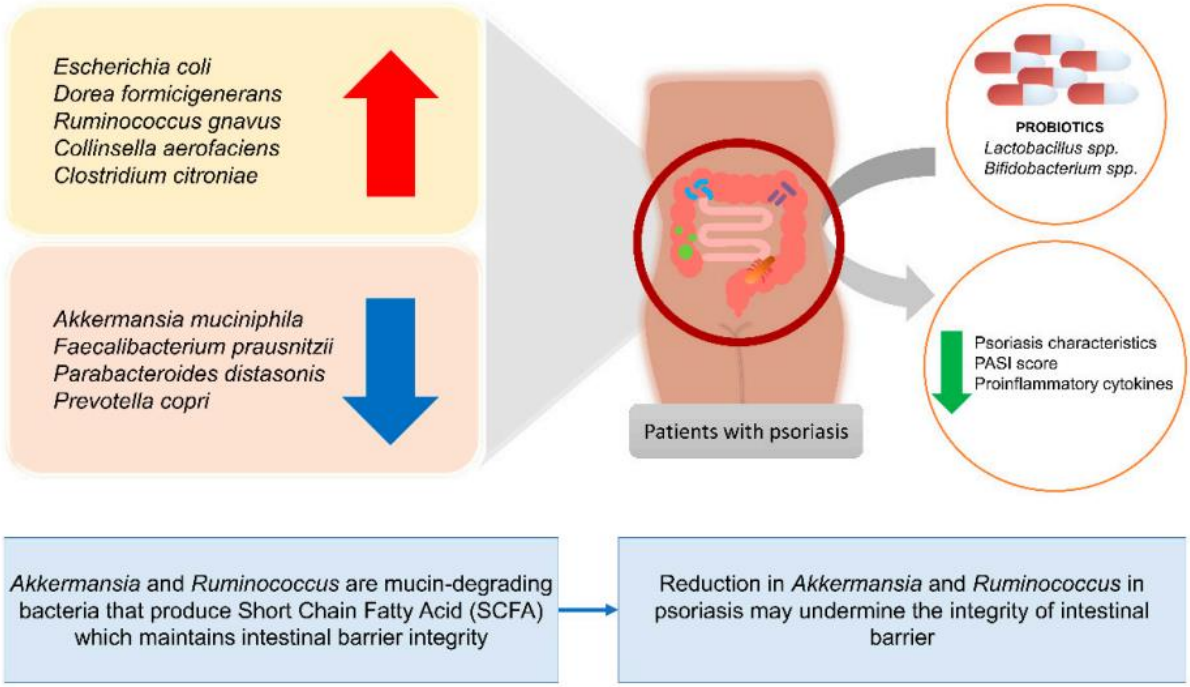

Figure 4.4 The association of gut dysbiosis in psoriasis [107]

Murine studies have shown that dietary supplementation with butyrate, a key SCFA, reverses or prevents insulin resistance. In contrast, decreased butyrate levels and increased acetate production have been linked to metabolic dysfunction. Similarly, fecal transplantation from obese to germ-free mice induced weight gain, suggesting that dysbiosis can drive obesity. The F/B ratio in psoriasis patients might also predispose them to diabetes and obesity, implicating gut microbiota in systemic metabolic disorders. [103]

Targeting the gut microbiome could potentially improve not only skin conditions but also metabolic health in psoriasis patients. Changes in the microbiota can trigger inflammation

through the activation of cytokines like IL-23, IL-17, and IL-22 while inhibiting Tregs and upregulating IFN-γ, leading to keratinocyte overproliferation. [103-105]

Additionally, studies consistently report reduced abundance of SCFA-producing bacteria such as *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Ruminococcus* in psoriasis patients. SCFAs exhibit anti-inflammatory properties by stabilizing the intestinal barrier, reducing oxidative stress, and inhibiting cytokine production. They also regulate immune pathways like NF-κB signaling and IL-6 production, helping to mitigate inflammation. [103-105]

In contrast, medium-chain fatty acids (MCFAs) show antibacterial properties and reduce intestinal inflammation by activating peroxisome proliferator-activated receptors (PPARs). However, MCFAs can also promote Th1 and Th17 differentiation while inhibiting Treg development, suggesting a complex interplay between gut microbes and immune responses. [103-105]

The gut microbiota's ability to metabolize tryptophan into immunomodulatory compounds like indole-3-aldehyde (IAld) further highlights its role in regulating local and systemic immune responses. IAld impacts IL-22 activity, protecting against excessive fungal growth like *Candida albicans*. These findings emphasize the potential of microbiome-based therapies to manage inflammatory diseases like psoriasis by targeting dysbiosis and restoring gut health. Addressing gut microbiome imbalances could improve skin health and reduce metabolic complications in psoriasis. For example, supplementation with *Bifidobacterium spp*. has been shown to lower serum levels of inflammatory markers, including TNF-α and CRP. Additionally, interventions targeting gut dysbiosis may help restore SCFA production, improve intestinal barrier function, and reduce systemic inflammation. [103-105]

4.1.5 Hidradenitis Suppurativa and the Microbiome

As stated in the previous chapters; Hidradenitis suppurativa (HS) is a chronic inflammatory skin condition characterized by recurrent, purulent lesions primarily affecting intertriginous regions such as the axillae, groin, and gluteal areas. The condition has a profound impact on quality of life, attributed to both physical symptoms, such as pain and malodorous discharge, and psychological effects, including depression and social isolation. The pathogenesis of HS is multifactorial, involving an interplay between genetic predisposition, environmental factors, immune dysregulation, and the microbiome. [107]

4.1.5.1 Chronic Inflammation and Microbial Imbalance in Hidradenitis Suppurativa (HS)

Traditionally, HS was thought to begin with follicular occlusion, which triggers an inflammatory cascade and secondary bacterial colonization. However, recent advances suggest a more intricate role for the skin and gut microbiomes in disease progression. Dysbiosis has been observed in HS, particularly in affected skin regions, where there is an overrepresentation of pathogenic bacteria such as *Staphylococcus aureus*, *Cutibacterium acnes*, and certain anaerobes such as coagulase-negative staphylococci (CNS). This imbalance is thought to exacerbate local inflammation by activating pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) on keratinocytes and immune cells. Moreover, microbial dysbiosis may extend beyond the skin. Emerging evidence suggests that gut microbiota alterations in HS patients could contribute to systemic inflammation. Reduced levels of beneficial taxa such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, both known for their anti-inflammatory properties, have been reported in HS, potentially linking gut microbiome imbalances to heightened immune activation and chronic inflammation. The microbiome also interacts with the host immune system through the production of microbial metabolites. For

instance, short-chain fatty acids (SCFAs), which are products of fermentation by certain gut bacteria, have been shown to support the integrity of epithelial barriers and modulate inflammatory responses. In HS, a deficiency in SCFA-producing bacteria may impair the skin and gut barriers, facilitating the translocation of antigens and promoting systemic inflammation. [108-110]

Concerning the mechanisms, there are several different hypotheses. A recent research proposed that a high-fat diet (HFD) triggers dysbiosis, leading to systemic inflammation and elevated levels of homocysteine (hyperhomocysteinemia, HHcy) in genetically or metabolically predisposed individuals. This dietary imbalance promotes the release of inflammatory cytokines, including IL-6, IL-1β, TNF-α, and IL-17. The resultant dysbiosis-induced inflammation exacerbates immune system dysregulation, further amplifying the inflammatory response. [108-110]

Additionally, HFD-induced dysbiosis disrupts 1-carbon metabolism, a key pathway in amino acid and methylation processes. This disruption is linked to the upregulation of matrix metalloproteinases (MMPs), specifically MMP-2, MMP-9, and MMP-8. These enzymes play a pivotal role in degrading extracellular matrix components, driving tissue remodeling and contributing to the formation and persistence of lesions and inflammatory tracts.[108-110]

The combined effects of heightened inflammation, immune dysregulation, and altered tissue matrix dynamics highlight the complex interplay between diet, microbiome balance, and metabolic processes in the development and progression of chronic inflammatory conditions. Targeting these pathways through dietary intervention and microbiome modulation may provide promising therapeutic avenues. These insights into the microbiome's involvement in HS have important implications for treatment. Strategies targeting microbial composition, such

as probiotics, prebiotics, and microbiota transplantation, are being explored as adjunct therapies. Understanding the interaction between microbial dysbiosis and immune dysregulation could pave the way for more personalized therapeutic approaches, aimed not only at alleviating symptoms but also at addressing the underlying causes of inflammation in HS.[108]

4.1.5.2 Biofilm Formation and Immune Response

Biofilm formation by bacteria such as *Staphylococcus aureus* is a key feature in HS pathogenesis. Biofilms protect bacteria from immune clearance and antibiotics, perpetuating inflammation. These biofilm-associated bacteria can activate innate immune pathways, leading to the release of pro-inflammatory cytokines and recruitment of neutrophils, further exacerbating tissue damage.[111-113]

Microscopic examination of HS lesions frequently reveals inflammatory infiltrates, which are often associated with biofilm presence, particularly in advanced stages of the disease. While HS is not classified as an infectious disease, certain studies have identified slow-growing microbial agents within its lesions.[113]

Research into the microbiome of sinus tracts in patients with moderate to severe HS indicates a predominance of anaerobic bacteria, including species such as *Prevotella* and *Porphyromonas*. The accumulation of intradermal corneocytes and hair fragments creates an ideal environment for biofilm formation by commensal bacteria. This phenomenon is further supported by the recurrent detection of anaerobic species within HS lesions, thriving in the anoxic conditions fostered by deep-seated nodules, dilated hair follicles, and sinus tracts. Studies have shown that biofilms are present in more than 65 % of HS lesions, yet their identification can be challenging with conventional culturing methods, which typically target

planktonic bacterial states. This limitation may explain why biofilms are often associated with chronic lesions.[113]

The standard treatment for HS lesions relies on tetracyclines, while second-line therapies involve a combination of clindamycin and rifampicin. This combination has demonstrated synergistic effects, reducing the risk of antibiotic resistance. However, when these antibiotics are used as monotherapy, resistance rates have been reported as 66% for clindamycin and 69% for rifampicin. Third-line treatments, such as dapsone, may be used for mild to moderate cases, though evidence supporting its effectiveness remains limited. Severe cases may require alternative antibiotics such as metronidazole or ertapenem, with the latter showing resistance rates of less than 1%.[113]

The frequent flare-ups experienced by HS patients can be partially attributed to biofilm formation, which complicates treatment outcomes and perpetuates inflammation. Addressing biofilm-related challenges in HS is essential for improving therapeutic strategies and reducing the burden of the disease on affected individuals.[113]

4.1.6 Atopic Dermatitis and the Skin Microbiome

As previously stated, atopic dermatitis (AD) atopic dermatitis (AD) is a chronic inflammatory skin condition associated with an imbalance in the skin microbiome. Recent advancements in research, highlight the significant role of gut microbiota disturbances in triggering abnormal immune responses, which can contribute to skin inflammation and potentially play a critical role in the development of AD. Emerging evidence suggests that gut microbiota actively regulates immune processes, which are intricately linked to the onset and progression of AD. The condition is commonly associated with immune system abnormalities, particularly

dysregulation of the Th2 inflammatory response. These immune irregularities often result in reduced diversity and stability within the gut microbiome, characterized by diminished populations of beneficial bacteria and an overgrowth of opportunistic pathogens. Consequently, understanding the specific alterations in the gut microbiota of individuals with AD, as well as the underlying mechanisms driving these changes, is essential for advancing potential treatments that target the gut microbiome.[114,115]

The multifaceted pathogenesis of AD involves various contributors, including immune dysregulation, impaired skin barrier integrity, skin microbiota imbalances, and environmental influences. Despite this, the precise interplay of these factors remains incompletely understood. Recent findings reveal a strong association between gut microbiota dysbiosis and allergic conditions such as eczema, asthma, and AD. Maintaining a stable gut microbiome is pivotal in modulating immune function, reinforcing the skin barrier, and regulating the neuroendocrine system, thereby influencing AD outcomes.[114]

Clinical investigations have identified notable alterations in the gut microbiota of individuals with AD. For example, increased levels of *Staphylococcus aureus*, *Escherichia coli*, and *Clostridium* species, coupled with a significant reduction in *Bifidobacterium bifidum*, have been observed in patients compared to healthy controls. Furthermore, longitudinal studies suggest that children with AD exhibit reduced gut microbiota diversity, with lower proportions of *Bacteroides* at one month and *Proteus* at twelve months of age. Probiotic interventions have demonstrated promise in restoring microbial balance and alleviating pruritus in AD patients, underscoring their therapeutic potential in managing the disease. [114,115] These therapeutic elements have been discussed in the further sections.

4.1.6.1 Dysbiosis and Atopic Dermatitis

Studies indicate that nearly 90% of individuals with AD have *Staphylococcus aureus* present on their lesional skin, whereas this bacterium is rarely found in the skin of healthy individuals. Furthermore, higher levels of *S. aureus* in affected skin are correlated with disease exacerbations. Longitudinal analysis using 16S rRNA gene sequencing of skin samples from children with AD has demonstrated significant shifts in the bacterial community during disease flares, with a notable increase in the proportion of *S. aureus*.[116]

Additionally, during AD flare-ups, untreated patients show a marked increase in *Staphylococcus* and *Corynebacterium* species, accompanied by a reduction in bacterial diversity. This reduction includes decreased populations of *Propionibacterium*, *Streptococcus*, *Acinetobacter*, and *Corynebacterium* species. Following treatment and clinical remission, bacterial diversity tends to increase, characterized by higher levels of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Propionibacterium* species, suggesting a connection between microbial diversity and disease severity.[117]

A meta-analysis of 95 observational studies reported that *S. aureus* colonized approximately 70% of lesional skin, 39% of non-lesional skin, 62% of nasal samples in AD patients, and only 3% in non-atopic individuals. Additionally, another study demonstrated that the natural presence of *S. aureus* and *Corynebacterium bovis* colonization triggered eczematous dermatitis in a mouse model, mimicking the inflammation observed in AD patients. These findings underscore the role of dysbiosis as a contributing factor in driving eczematous inflammation and exacerbating AD.[116]

Besides from the skin dysbiosis, gut dysbiosis could also have an impact in AD. Among AD patients experiencing gastrointestinal symptoms, a decrease in *Prevotella copri* and an increase in *Bacteroides* levels are linked to the exacerbation of symptoms. This subgroup also

demonstrates distinct gut microbiota characteristics, with lower diversity and greater uniformity compared to AD patients without gastrointestinal symptoms or healthy controls. Specifically, patients with epigastric rigidity show an increased abundance of *Bacteroides* and decreased levels of *Prevotella*. [118]

In adults with late-onset AD, the predominant genus is *Escherichia-Shigella*, accompanied by significantly reduced levels of *Agathobacter* and *Dorea*. This group also displays a marked increase in the *Bacteroides pectinophilus* group compared to other AD subtypes. Conversely, persistent AD in adults is associated with a predominance of *Faecalobacterium*, while the microbiota of healthy adults is dominated by *Subdoligranulum*. The microbiota composition varies significantly among individuals with skin allergies (AD and urticaria) compared to those with allergic rhinitis. This highlights the presence of distinct gut-skin and gut-nose axes. Common dysbiosis features in allergic diseases include an increase in *Firmicutes* species, members of the *Bacteroidales* order, and the *Ruminococcaceae* family (*Clostridia*). [118]

Adults with allergies, such as nut and pollen sensitivities, show reduced gut microbial diversity, decreased *Clostridiales*, and increased *Bacteroidales*. However, this altered composition does not appear to be significantly associated with AD. Patients with AD exhibit reduced microbial diversity in the skin and oral cavity, which correlates with disease severity. Interestingly, these differences are not observed in fecal microbiota, indicating a localized effect of dysbiosis in AD. Adults with intractable AD show lower levels of fecal polyamines, such as putrescine and spermidine, compared to healthy controls. This reduction correlates with an increased abundance of *Enterobacteriaceae*, a bacterial family known to disrupt the intestinal barrier and affect polyamine absorption. Genera more commonly associated with AD in adults include

Bacteroides (*Bacteroidales* order), *Escherichia-Shigella* (*Enterobacteriaceae* family), and *Clostridium perfringens*.[118]

In contrast, healthy adults have a higher prevalence of "protection-associated" bacteria, including *Prevotella*, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Clostridium* (*cluster IV and subcluster XIV*), and *Faecalibacterium prausnitzii*. These bacteria are known for producing short-chain fatty acids (SCFAs) and polyamines, supporting a diverse and robust gut microbiome. [118]

4.1.6.2 Mechanism of Skin-Gut Dysbiosis and Atopic Dermatitis

Traditionally, AD has been viewed as a childhood condition driven by an overactive T-helper-2 (Th2) immune response, which leads to heightened IgE-mediated sensitivity to allergens. However, the pathophysiology of AD is now understood to be more intricate than previously thought. One contributing factor is the compromised epidermal barrier, often linked to genetic mutations in keratinocyte proteins like filaggrin, which heighten vulnerability to AD.[116]

The increased penetration of *S. aureus* into the skin correlates with a Th2 immune response, characterized by heightened expression of IL-4, IL-13, IL-17, and thymic stromal lymphopoietin, alongside reduced antimicrobial peptide (AMP) levels. Th2 cytokines have been shown to directly suppress AMP induction in the skin. Although mutations in the FLG gene are a well-known risk factor for atopic dermatitis (AD), only a minority of individuals with FLG mutations develop the condition. Other epidermal proteins, including envoplakin, periplakin, and involucrin, may also regulate bacterial penetration and contribute to inflammatory responses. These skin barrier defects increase susceptibility to AD by allowing microbial entry into the dermis. Restoring the skin barrier with optimized lipid mixtures

containing cholesterol, ceramide, and free fatty acids has been shown to reduce *S. aureus* colonization, decrease proinflammatory cytokines, and enhance AMP expression, such as cathelicidin and β-defensins, in experimental models. Human skin actively limits microbial colonization by producing AMPs (e.g., cathelicidin and β-defensin), fatty acids, and reactive oxygen species, all of which inhibit bacterial growth. Dysregulation of these defense systems can lead to dysbiosis and increased infection risk. In AD, *S. aureus* colonization is partly attributed to reduced production of AMPs, which are significantly less abundant in AD skin compared to other inflammatory skin conditions like psoriasis or wounds. Th2 cytokines, such as IL-4 and IL-13, further suppress cathelicidin production in keratinocytes. Additionally, *S. aureus* secretes aureolysin, a metalloprotease that degrades cathelicidin into inactive fragments, facilitating bacterial survival.[119,120]

Certain coagulase-negative staphylococci (CNS) strains in the skin microbiome provide antimicrobial defense. For example, *Staphylococcus epidermidis* produces peptides such as PSMγ and PSMδ, which disrupt bacterial membranes, mimicking the action of human AMPs. Lantibiotics, cyclic AMPs produced by *Staphylococcus hominis* and other CNS, also synergize with host AMPs to inhibit pathogens like *S. aureus*, *Streptococcus pyogenes*, and *Escherichia coli*. These antimicrobials exhibit selective activity, targeting pathogenic bacteria while preserving commensal microbes, thereby maintaining microbial ecosystem balance. Some CNS strains also produce proteases and peptides that degrade S. aureus biofilms or inhibit its quorum sensing system, reducing its virulence.[119]

Studies have shown that the skin of AD patients lacks protective CNS strains that inhibit *S. aureus*. Individuals heavily colonized by *S. aureus* have lower levels of CNS with antimicrobial activity. Additionally, reduced CNS colonization during infancy is linked to a higher risk of

developing AD later in life. Experimental data suggest that early-life exposure to beneficial CNS strains can enhance regulatory T cell responses, potentially protecting against AD development.[119]

Specific microbiota signatures associated with dysbiosis in AD include an elevated *Firmicutes/Bacteroidetes* ratio and reduced microbial diversity, particularly in children. In adults, a decrease in SCFA-producing genera such as *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Bacteroides*, and *Streptococcus* has been noted, alongside a diminished capacity for immunotolerance due to reduced regulatory T cell (Treg) induction.[117]

The gut microbiome plays a vital role in activating dendritic cells and influencing regulatory T cell pathways (Th1, Th2, Th17, and Treg), thereby contributing to immune tolerance and regulation. This interplay between the gut, immune system, and skin is described as the "gut-skin axis."[118]

4.1.7 Inflammatory Bowel Disease: Ulcerative Colitis and Crohn's Disease

The relationship between genetic susceptibility to IBD and the gut microbiota has been extensively explored, particularly through high-throughput sequencing techniques. IBD is understood to result from complex interactions between host genetics, microbial factors, an impaired mucosal barrier, and an abnormal immune response. Disruption of microbial homeostasis creates an environment that fosters the colonization and invasion of opportunistic pathogens, heightening the risk of immune system activation and accelerating IBD progression. Identifying the specific pathogens involved in this process is essential for advancing early diagnostic capabilities and developing targeted interventions. [121,122]

IBD emerges as a multifactorial condition driven by the interplay of genetic predisposition, environmental influences, and the gut microbiome. Each factor plays a necessary but insufficient role independently in causing disease. Growing evidence highlights a bidirectional relationship between IBD progression and alterations in the gut microbiota's composition and functionality. This dynamic relationship underscores the critical importance of studying host-microbe interactions to uncover key mechanisms of disease pathogenesis and to explore potential microbiome-based therapeutic strategies.[122,123]

Over the past decade, genome-wide association studies and other genetic analyses have revealed that IBD is linked to loci associated with aberrant immune responses to intestinal microbiota. Concurrently, studies profiling the intestinal microbiome have identified characteristic compositional changes in microbial communities that correlate with disease pathogenesis. These findings reinforce the hypothesis that IBD arises from dysregulated interactions between intestinal microbes and the mucosal immune system.[121-123]

Advances in microbiome research technologies, including metagenomics and metabolomics, have provided deeper insights into the mechanisms underlying intestinal microbiota dysfunction in IBD. Early exploratory studies using 16S ribosomal RNA sequencing established associations between specific microbial taxa and IBD. More recent approaches have adopted a functional perspective, examining microbiota activity and metabolic pathways to elucidate their contributions to disease progression.[122,123]

Continued progress in understanding the microbiome’s role in IBD requires innovative study designs and methodologies. Future research should aim to integrate multi-omics approaches to characterize microbial communities at both the metabolic and pathway levels. This will enhance our understanding of host-microbe interactions and their implications not only for IBD but also

for autoimmune and other inflammatory diseases. Such advancements will pave the way for more precise diagnostic tools and microbiome-targeted therapies to improve patient outcomes.[121-124]

4.1.7.1 The Gut Microbiome in IBD: Ulcerative Colitis (UC) and Crohn's Disease (CD)

Inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic gastrointestinal inflammation. Dysbiosis in the gut microbiome is a hallmark of IBD, with reduced microbial diversity and a shift towards pro-inflammatory taxa such as *Enterobacteriaceae* and *Bacteroides*. Research involving human participants has established that individuals with IBD exhibit a gut microbiota composition distinct from that of healthy individuals. Furthermore, variations exist in the gut microbiota profiles between ulcerative colitis (UC) and Crohn's disease (CD) patients. Global analyses of IBD-associated gut microbiota have consistently identified dysbiosis, characterized by imbalances in specific bacterial populations.[123-127]

One longitudinal study by Morgan et al. observed that patients with IBD display a unique microbial profile compared to healthy controls. A significant reduction in the abundance of *Roseburia* and *Phascolarctobacterium* was noted, while *Clostridium* levels were elevated in patients with UC or CD. *Roseburia* is linked to the production of regulatory T cells, which exhibit anti-inflammatory properties in the gut. Similarly, *Phascolarctobacterium*, which produces the SCFA propionic acid, was found to be decreased, potentially impairing anti-inflammatory pathways and exacerbating IBD symptoms.[124-126]

In healthy individuals, the *Clostridium leptum* group comprises 16–25% of the gut microbiota. However, its diversity and richness are altered in IBD patients, even during remission phases.

This group, also known as Clostridial cluster IV, includes beneficial bacteria such as *Faecalibacterium prausnitzii*, *Eubacterium*, and *Ruminococcus*. Notably, *F. prausnitzii* abundance is reduced in CD patients and mouse models of colitis, suggesting its critical role in maintaining gut health. Low levels of *F. prausnitzii* are associated with disease onset and recurrence, highlighting its potential as a probiotic therapy to restore microbial balance and alleviate symptoms.[124,125]

Twin studies have further demonstrated the environmental influence on gut microbiota composition in IBD. In cases where one twin had IBD while the other was healthy, microbial differences were evident, despite shared genetics. These findings suggest that environmental factors, including gut microbiota alterations, may play a more significant role than genetic predisposition in IBD pathogenesis. Multi-omics approaches, incorporating metagenomics, transcriptomics, and metabolomics, have identified specific microbial and metabolite differences during active and remission phases of UC and CD. For instance, metabolites such as nicotinuric acid, taurine, and acylcarnitines are elevated in IBD patients, with taurine linked to inflammasome activation and inflammation. [124,125]

Pathobionts, including *Escherichia coli* and *Enterococcus faecium*, are often enriched in IBD patients. Adherent-invasive *E. coli (AIEC)*, commonly found in CD ileal mucosa, promotes inflammation by adhering to epithelial cells, penetrating the lamina propria, and evading autophagic and immune responses. AIEC also secretes colibactin, a genotoxin linked to tumorigenesis in mouse models. Other bacterial species, such as enterotoxigenic *Bacteroides fragilis* (ETBF) and *Campylobacter concisus*, contribute to intestinal inflammation by disrupting epithelial barriers and triggering immune responses. For instance, ETBF increases

colonic permeability and promotes IL-17 production, while *C. concisus* damages tight junctions and induces pro-inflammatory cytokines like TNF-α. [123-125]

Fungal dysbiosis, or alterations in the gut mycobiome, is another hallmark of IBD. Patients exhibit a higher Basidiomycota-to-Ascomycota ratio, with increased abundance of *Candida* species, particularly *C. albicans* and *C. tropicalis*. These fungi exacerbate colitis in mouse models through immune mechanisms involving CARD9 signaling. Conversely, beneficial fungi such as *Saccharomyces cerevisiae* have shown protective effects in experimental models of UC but may also contribute to disease under specific conditions. [123-125]

The virome, predominantly composed of bacteriophages, also undergoes shifts in IBD. Increased abundance of *Caudovirales phages* has been observed in both pediatric and adult IBD cohorts, with certain phages linked to heightened IFN-γ production and intestinal inflammation. These findings suggest a potential role for bacteriophages in modulating bacterial populations and influencing disease outcomes. [123-125]

The interplay between the gut microbiota, their metabolites, and host immune responses underpins IBD pathogenesis. Emerging evidence highlights the potential of personalized therapies targeting microbial dysbiosis to manage IBD. Larger studies and molecular approaches are needed to identify predictive biomarkers and develop targeted interventions that restore microbial balance and alleviate inflammation. [123-127]

4.1.7.2 Microbial Triggers of Inflammation in UC and CD

Microbial dysbiosis in IBD promotes inflammation through various mechanisms, including disruption of the intestinal barrier, activation of immune responses via toll-like receptors (TLRs), and altered metabolite production. Short-chain fatty acids (SCFAs), primarily

produced through the fermentation of dietary fibers by gut bacteria, are crucial for maintaining intestinal homeostasis. Bacteroidetes predominantly produce propionate and acetate, while Firmicutes are the main producers of butyrate. SCFAs serve as key energy sources for intestinal epithelial cells and also bind to G-protein coupled receptors (GPCRs), initiating signaling pathways that promote mucosal healing, histone deacetylation, and gene expression. They are vital for immune modulation, influencing T regulatory (Treg) cell development, cytokine production, and anti-inflammatory processes. Additionally, SCFAs regulate the gut microbial ecosystem by creating an environment that selects for specific microbial communities. However, disruptions in SCFA production often accompany dysbiosis. For instance, reduced butyrate synthesis is a hallmark of gut microbiota imbalances and is commonly observed in inflammatory bowel disease (IBD). Diminished butyrate not only compromises epithelial barrier function but also reduces macrophage differentiation and antimicrobial peptide (AMP) production. This creates a feedback loop, where an impaired barrier facilitates pathogen overgrowth, exacerbating inflammation and further altering microbial populations.[122-125]

The production and metabolism of bile acids provide another example of the profound effects of dysbiosis. Primary bile acids synthesized by the liver are transformed by gut microbes into secondary bile acids (SBAs) in the colon, a process essential for maintaining bile acid homeostasis and lipid metabolism. Dysbiosis, characterized by reduced microbial diversity and function, often impairs this transformation. In IBD, inflammation and malabsorption further disrupt the bile acid pool, leading to decreased SBA production. This dysregulation alters the intestinal milieu, creating conditions conducive to infections such as *Clostridioides difficile*. Reduced SBAs fail to inhibit the germination of C. difficile spores, while inflammation-driven alterations in bile acid reabsorption amplify this vulnerability. Additionally, SBAs modulate immune responses by promoting the differentiation of RORγ-expressing Treg cells.

Experimental evidence highlights that the loss of microbial bile acid metabolic pathways decreases these immune-regulating populations. Specific SBA derivatives, such as 3-oxoLCA and isoalloLCA, influence immune balance by inhibiting pro-inflammatory Th17 cells and promoting Treg cells. Dysbiosis, by reducing the microbial genes and enzymes required for SBA production, exacerbates inflammation and gut dysfunction. [122-125]

Microbial metabolism of aromatic amino acids further illustrates how dysbiosis impacts host health. Tryptophan, for example, is metabolized by gut bacteria into bioactive compounds like indoleacrylic acid, indole-3-propionic acid, and indole-3-aldehyde. These metabolites activate the aryl hydrocarbon receptor (AHR), a regulator of gene expression critical for maintaining epithelial barrier integrity and immune cell differentiation. Dysbiosis disrupts this metabolic pathway, leading to reduced levels of tryptophan metabolites in the systemic circulation. In IBD, this impairment is especially pronounced, with decreased tryptophan availability correlating with increased disease severity. Animal studies have shown that microbiota lacking tryptophan metabolism exacerbate colitis in recipients, while supplementation with tryptophan or its metabolites can alleviate inflammation. This highlights the potential therapeutic value of restoring microbial functions disrupted by dysbiosis. [122-125]

Sphingolipid metabolism provides another layer of insight into the mechanisms by which dysbiosis drives disease. Sphingolipids, produced by both host cells and gut microbes, are critical for regulating inflammation and immune responses. Host-derived sphingolipids are involved in signaling pathways linked to IBD pathogenesis, while microbial-derived sphingolipids serve protective roles. For instance, sphingolipids produced by Bacteroides inhibit the proliferation of invariant natural killer T (iNKT) cells, reducing intestinal inflammation. Dysbiosis, marked by diminished microbial sphingolipid production, disrupts this balance. In murine models, the absence of microbial sphingolipids leads to intestinal

inflammation and alterations in host ceramide pools. This shift may trigger compensatory increases in host-derived sphingolipids, which can exacerbate inflammatory pathways and further impair gut barrier function. [122-129]

Dysbiosis disrupts these metabolic processes through several mechanisms, including the overgrowth of pathogenic microbes, depletion of beneficial species, and reduced functional diversity of the microbiota. These changes impair critical microbial enzymatic pathways, altering the production of metabolites essential for maintaining gut homeostasis. Inflammation, a common consequence of dysbiosis, creates an oxidative and hostile environment that favors facultative anaerobes over obligate anaerobes, further skewing microbial composition. This ecological imbalance perpetuates a vicious cycle, where the loss of beneficial microbes and metabolites exacerbates inflammation, driving further microbial shifts. Together, these disruptions compromise the host's immune regulation, barrier function, and metabolic homeostasis, underscoring the profound impact of dysbiosis on health and disease. [122-130]

4.1.7.3 Role of Diet, Antibiotics, and Microbiome-Targeted Therapies

Diet and antibiotics significantly influence the gut microbiome in IBD. High-fiber diets rich in prebiotics can promote beneficial bacteria, while antibiotics may exacerbate dysbiosis. Microbiome-targeted therapies, including probiotics, prebiotics, and fecal microbiota transplantation (FMT), are being explored as potential treatments to restore microbial balance and mitigate inflammation. A detailed section has been dedicated to the treatment of microbiome in IMIDs (refer to chapter 4 section 4.1.8). [128,131]

4.1.8 Microbiome-Based Therapies in Immune-Mediated Diseases

Therapeutic approaches to modify the intestinal microbiome typically involve dietary adjustments, the use of prebiotics, probiotics, antibiotics, or, more recently, fecal microbiota transplantation (FMT). When it comes to IMIDs the same approaches could be applied, but they are limited. Especially For IBD there are more specific approaches such as nucleic acid based therapies or metabolite based or taxonomy based therapies (Figure 4.5) The specific targets and efficacy of these interventions remain incompletely understood and have only recently been explored in scientific literature. The goal of these therapies is to shift microbial communities from states associated with dysbiosis to those linked with health. While some studies have shown clinical improvements following treatment with various microbial formulations, interpreting these outcomes is complex. Changes in the microbiome do not always result in clinical improvement, clinical benefits can occur without detectable microbiome changes, and alterations in microbial composition may have no lasting impact on clinical symptoms.[128,131-146]

The gastrointestinal microbiome plays a critical role in maintaining mammalian health by participating in essential physiological functions and influencing host development. Disruptions in gut microbial populations have been linked to a range of gastrointestinal and immune mediated inflammatory diseases, positioning the intestinal microbiome as a promising diagnostic and therapeutic target. Despite advances in DNA sequencing and computational methods that have transformed microbiome research, many key questions remain unresolved. Future studies should focus on uncovering the mechanisms driving microbiome-host interactions, understanding how the microbiome develops and matures during different life

stages, clarifying its role in disease processes, and evaluating the potential of diagnostic tools and therapeutic strategies to address conditions associated with intestinal dysbiosis.[131-146]

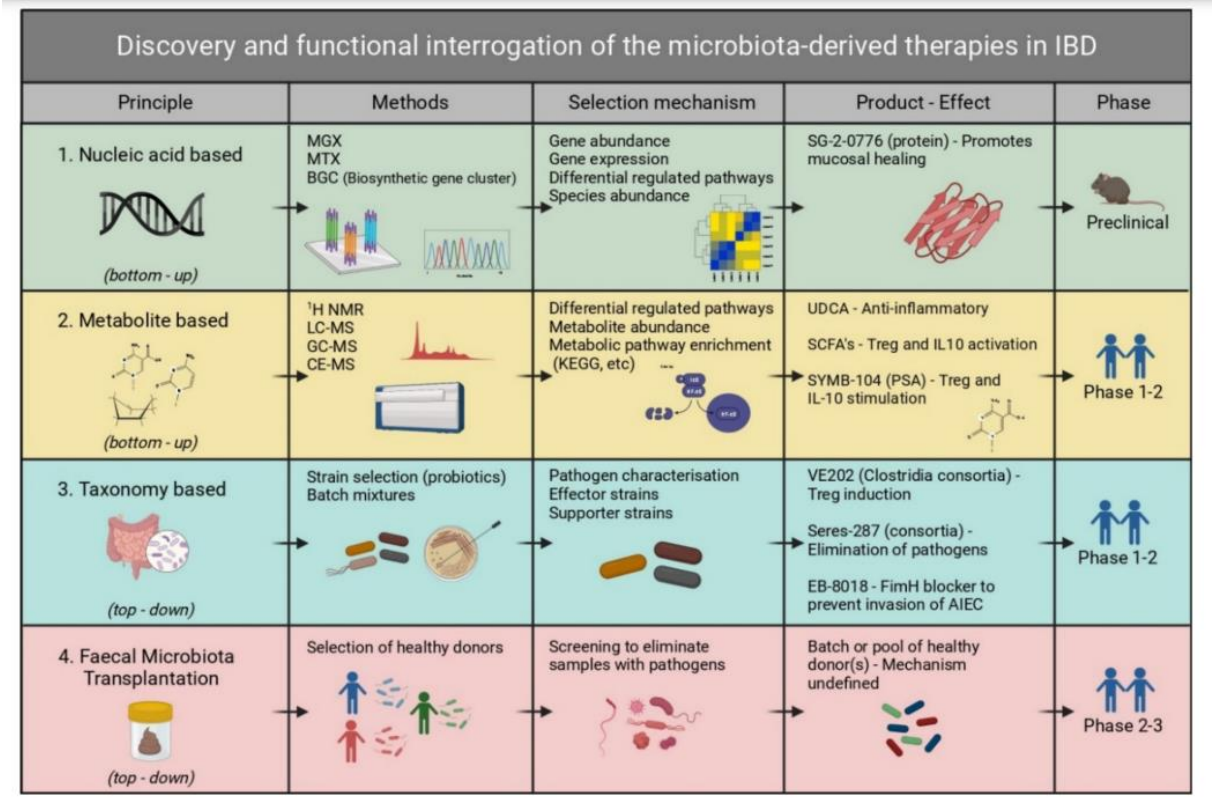

Figure 4.5 Microbiome based therapies in IBD [134]

4.1.8.1 Current therapeutic approaches: nutrition, prebiotics, probiotics, fecal microbiota transplantation (FMT), and microbiome-directed drugs

Diets high in fats and carbohydrates and low in fruits and vegetables are strongly associated with an increased risk of metabolic disorders. Research indicates that diet significantly impacts microbiota composition, accounting for approximately over 55% of its variation compared to only 12% for genetic factors. Studies have shown that prolonged consumption of diets rich in fats and sugars induces dysbiosis, leading to an increased abundance of species such as *Bacteroides spp.* and *Ruminococcus torques*. Similarly, diets high in saturated fats but low in

polyunsaturated fats—often sourced from vegetable oils—have been linked to dysbiosis and an overgrowth of *Bilophila wadsworthia*. This, in turn, triggers inflammation mediated by Th1 cells and results in colitis, as observed in mouse studies.[131]

Comparative dietary analyses highlight the distinct differences between Western and Eastern dietary patterns. The Western diet, which is rich in fats, refined carbohydrates, and animal proteins, is associated with a higher prevalence of *Bacteroides spp.* in the gut microbiota. In contrast, the plant-based Eastern diet, which includes carbohydrates from sources like rice, vegetables, and fruits, is linked to a greater abundance of *Prevotella spp.* Animal protein and fats promote the growth of *Bacteroides spp.*, while diets rich in simple carbohydrates and fiber encourage the proliferation of *Prevotella spp.*. [131]

The fermentation of dietary carbohydrates by gut bacteria produces short-chain fatty acids (SCFAs), which support intestinal health. However, protein fermentation generates potentially harmful metabolites, including organic acids, phenolic compounds, indoles, and ammonia, which can have toxic effects on the gut. Diets high in fats and sugars have been shown to disrupt the gut microbiota, resulting in dysbiosis and increased endotoxin production. Dysbiosis compromises the integrity of the intestinal mucosa, making it thinner and more permeable to pathogens and antigens, leading to chronic low-grade inflammation. On the other hand, fiber-rich diets promote a healthy gut by lowering intestinal pH and inhibiting the growth of pathogenic bacteria such as *Escherichia coli* and other *Enterobacteriaceae*. These findings emphasize the close relationship between diet quality, microbiota composition, and gut health. [131]

The role of a Western diet—characterized by high levels of refined carbohydrates, simple sugars, saturated fats, and omega-6 fatty acids— has been reviewed in triggering conditions

like ulcerative colitis (UC) and Crohn's disease (CD). Excessive consumption of animal proteins has been particularly linked to an increased risk of CD, while diets rich in fruits and vegetables appear to have a protective effect. In patients with CD, microbiota composition is altered, with increased levels of *Proteobacteria* and *Bifidobacteria* and decreased levels of *Firmicutes*. For UC, high intake of refined carbohydrates, simple sugars, and fatty acids is associated with an elevated risk. Moreover, imbalances in polyunsaturated fatty acid (PUFA) ratios have been linked to increased susceptibility to both CD and UC. [131]

Vitamin D deficiency has also been implicated in inflammatory bowel disease (IBD) pathogenesis. In mouse models, vitamin D deficiency has been shown to exacerbate colitis, while supplementation exerts anti-inflammatory effects by suppressing pro-inflammatory gene expression, such as TNF genes. In addition, PUFAs and conjugated linoleic acid (CLA) have demonstrated anti-inflammatory properties in IBD management. These compounds reduce the production of interferon-γ and prostaglandin E2 and modulate T-cell activity, offering therapeutic benefits. [131]

The Mediterranean diet is widely regarded as beneficial for preventing and managing intestinal diseases. This dietary pattern emphasizes high consumption of fruits, vegetables, olive oil, oily fish, whole grains, and nuts, providing a rich source of fiber, antioxidants, and vitamins, as well as mono- and polyunsaturated fatty acids. Structured around a standardized food pyramid, this diet supports gut health and overall well-being. Recent research suggests that incorporating probiotics, such as *Lactobacillus rhamnosus*, alongside a Mediterranean diet can help restore eubiosis by positively altering microbiota composition. Prebiotic foods, particularly those containing soluble fiber like inulin, also contribute to maintaining intestinal balance. Inulin fermentation by gut bacteria produces SCFAs, such as butyrate, which have beneficial effects

on colonocytes and exhibit anti-inflammatory properties by suppressing pro-inflammatory factors like NF-κB. These findings highlight the potential of dietary interventions in managing IBD symptoms and promoting gut health. [131]

The therapeutic potential of prebiotic supplementation, particularly through the inclusion of plant-based polysaccharides, has gained recognition as a strategy to modify the intestinal microbiome. Common prebiotics include inulin, non-starch polysaccharides found in cereal grains and certain seaweeds, disaccharides like lactulose, and polysaccharides such as fructooligosaccharides (FOS) and galactooligosaccharides. These compounds are fermented by colonic bacteria, producing short-chain fatty acids (SCFAs) that provide vital nutrients for the intestinal epithelium. SCFAs also help regulate inflammation by inducing anti-inflammatory T regulatory (Treg) cells and reducing luminal pH, which can inhibit the growth of specific pathogens. Additionally, prebiotics may protect the intestinal lining by enhancing the mucus layer, elongating microvilli, increasing epithelial cell numbers, and preventing the adhesion of harmful bacteria. Certain SCFAs, such as propionate, are also known to promote Treg cell generation in the peripheral immune system. Recent animal studies have shown that a fiber-deficient diet leads to the degradation of the protective mucosal layer by commensal bacteria, making the host more susceptible to pathogen invasion. The effects of prebiotics have been extensively studied in animals. In one investigation, chicory root, a source of inulin, improved fecal consistency, increased Bifidobacteria populations, and reduced *Clostridium perfringens* levels in healthy dogs. A meta-analysis involving multiple studies revealed that prebiotic supplementation leads to a dose-dependent increase in fecal SCFA concentrations, as well as an increase in beneficial bacteria like *Bifidobacteria* and *Lactobacilli*, independent of dietary composition. Similar benefits have been observed in cats, where pectin administration raised

Lactobacilli levels and fecal SCFA concentrations, while FOS supplementation increased *Bifidobacteria*, reduced *Escherichia coli*, and elevated fecal butyrate levels.[128]

Probiotics, on the other hand, are live microbial formulations that confer health benefits when consumed in sufficient quantities. Proposed mechanisms for their efficacy include reducing intestinal permeability through tight junction protein enhancement, stimulating mucin secretion by goblet cells, increasing defensin production to prevent pathogen colonization, generating SCFAs, and modulating immune responses to promote tolerance to commensal microbes while maintaining defense against pathogens. Even nonviable probiotics may provide benefits by interacting with the mucus layer and activating immune responses. For instance, capsular polysaccharides from *Bacteroides fragilis* have been shown to activate Tregs and reduce colitis severity in mouse models. Common probiotic strains include Lactobacillus and Bifidobacterium species, as well as *Bacillus*, *Streptococcus*, and the yeast *Saccharomyces boulardii*.[128]

To remain effective, probiotics must survive gastric acidity and bile exposure to colonize the intestines. Some studies have shown that probiotic species are detectable in the gastrointestinal tract only during supplementation and not after discontinuation, indicating that their colonization is often transient but still beneficial. Research dating back to the early 20th century has demonstrated the therapeutic potential of probiotics, with more recent studies highlighting specific strains like *Bifidobacterium longum* for reducing pathogenic infections. [128]

The probiotic mixture VSL#3, which contains lyophilized strains of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus salivarius*, has been studied extensively in both humans and animals. In clinical trials, VSL#3 has been shown to prolong remission in ulcerative colitis patients and improve symptoms in children with the condition. Probiotics have also been

associated with shorter durations of acute gastroenteritis, reduced symptoms of rotavirus diarrhea, and lower incidences of antibiotic-associated diarrhea in humans. [128,130]

Animal studies have further supported the efficacy of probiotics. In dogs with IBD , Saccharomyces boulardii improved clinical symptoms, while Lactobacillus supplementation reduced Enterobacteria levels and improved fecal microbiota. Probiotic combinations have also been shown to alleviate symptoms of acute gastroenteritis and enhance immune responses, such as increased IgA secretion. In puppies and shelter cats, Enterococcus faecium SF68 has reduced diarrhea incidence and improved fecal microbial balance. A synbiotic containing probiotics and prebiotics was found to improve fecal consistency and reduce diarrhea episodes in sled dogs. Moreover, in dogs with IBD, VSL#3 supplementation increased Treg cell markers and improved clinical outcomes compared to conventional treatments. These findings underscore the potential of prebiotics and probiotics as therapeutic tools to modulate the gut microbiome and improve health outcomes in both humans and animals.[128]

The effectiveness of fecal microbiota transplantation (FMT) in recurrent *Clostridioides difficile* infection (rCDI) has been extensively documented, with cure rates reaching approximately 90% in over 500 reported cases. Given that microbiota alterations are a hallmark of IBD, FMT has also emerged as a potential therapeutic option for IBD treatment. Early clinical observations in patients with UC and CD have shown promising results, including instances of long-term clinical remission, with some cases reporting both endoscopic and histological improvements. [136-139]

A meta-analysis of nine studies involving 122 patients (79 with UC, 39 with CD, and four with unclassified IBD) reported an overall remission rate of 36.2%. Interestingly, remission rates were higher among younger individuals (aged 7–20 years) and those with CD (64.1% and

60.5%, respectively), compared to UC patients, where remission was achieved in only 22% of cases. However, findings from smaller studies have been inconsistent. For example, a study of five moderate-to-severe UC patients who received FMT via a nasojejunal tube found no significant remission after 12 weeks, except for one patient who showed a partial improvement. Conversely, another study involving nine pediatric UC patients treated with daily FMT enemas for five consecutive days reported a much higher success rate, with seven achieving remission after one week and six maintaining remission after a month. [136-139]

In CD, evidence also highlights variability. A study involving 25 CD patients receiving regular FMT every three months reported clinical remission in 52% after three months, with sustained remission observed in fewer individuals over time (48% at six months, 32% at 12 months, and 22.7% at 18 months). Another trial using FMT via midgut for refractory CD in 30 patients found clinical remission rates as high as 76.7% at one month. However, significant variability in treatment protocols—such as differences in FMT dosage, delivery routes, and donor stool preparation—makes it challenging to draw definitive conclusions about FMT's efficacy and safety in IBD. [136-139]

Recent randomized controlled trials provide more insight. One study found that weekly FMT enemas over six weeks resulted in higher remission rates in UC patients compared to placebo (24% vs. 9%). Another trial compared donor stool FMT to autologous FMT in mild-to-severe UC patients but found no statistically significant differences in clinical or endoscopic remission. [136-139]

Despite the success of FMT in rCDI, the outcomes for IBD remain less definitive, emphasizing that IBD is more complex and multifactorial, extending beyond microbiota-driven pathogenesis. Furthermore, adverse events associated with FMT must be considered. Short-

term side effects, including mild gastrointestinal symptoms and fever, are relatively common but generally resolve within weeks. Severe complications, though rare, include infections from donor material, colonic perforation during colonoscopy, and, in rare cases, death due to unrelated comorbidities. [136-139]

Long-term safety data remain sparse, raising concerns about potential risks, such as inducing metabolic conditions like obesity or diabetes. For instance, a weight gain incident linked to FMT from an overweight donor highlights the need for cautious donor selection and long-term follow-up. Animal studies have also shown that microbiota transfer can influence conditions like colitis and obesity, further underscoring the complexity of gut microbial interactions. [136-139]

In summary, while FMT holds promise as a treatment for IBD, its role remains unclear. Standardized treatment protocols, robust randomized trials, and long-term safety assessments are critical to optimizing its therapeutic potential. Future research should also focus on isolating and characterizing beneficial microbial metabolites or bacterial strains, such as *Faecalibacterium prausnitzii*, which has demonstrated anti-inflammatory properties in preclinical studies. Such advancements may pave the way for tailored microbiota-based therapies that address both immune dysregulation and microbial imbalances in IBD.[136-139]

One of the most recent research projects investigating the potential involvement of Gram-negative bacteria in AD has identified *Roseomonas mucosa*, a commensal bacterium, as a key player. For the first time in 2018, first in human topical microbiome transplantation has been realized with *R.mucosa*. Strains of *R. mucosa* isolated from healthy individuals demonstrated beneficial effects in both murine and cell culture models of AD, whereas strains obtained from

AD patients exacerbated disease symptoms in these models. These findings suggest that microbiome-targeted therapies could offer a novel treatment approach for AD.[141]

To explore this hypothesis in a clinical setting, an open-label phase I/II study, known as the Beginning Assessment of Cutaneous Treatment Efficacy for Roseomonas in Atopic Dermatitis (BACTERiAD I/II), was conducted with 10 adult and 5 pediatric participants. Application of R. mucosa resulted in notable improvements, including reduced disease severity, decreased reliance on topical corticosteroids, and a decline in S. aureus colonization. No adverse effects or safety concerns were reported. Furthermore, the study examined bacterial metabolites and environmental exposures that may contribute to the microbial imbalances observed in AD, providing insights for future microbiome-based interventions. These preliminary findings support further investigation through a placebo-controlled trial to validate the therapeutic potential of R. mucosa in AD management.141

Another microbiome-based therapy in AD was to used *S. aureus* strains. We know that topical corticosteroids are a cornerstone of AD management due to their potent anti-inflammatory properties. However, their long-term use is associated with adverse effects, such as skin atrophy, and the potential for disease relapse upon discontinuation. To address these limitations, alternative approaches have been explored, particularly those targeting Staphylococcus aureus, a key factor in AD pathogenesis. Strategies to eliminate *S. aureus* have focused on its strain-specific characteristics. One such approach, bleach bath therapy using sodium hypochlorite, has been evaluated for its potential to reduce *S. aureus* colonization. While a randomized, double-blind study demonstrated some effectiveness, subsequent research found that bleach baths did not offer greater benefits compared to water baths alone in reducing corticosteroid and

antibiotic use. Further investigations are needed to determine the appropriate clinical indications for this therapy in AD management.[142]

More recent research has shifted focus from eradicating *S. aureus* to restoring microbial balance on the skin. Certain coagulase-negative *Staphylococcus* species, such as *Staphylococcus epidermidis* and *Staphylococcus hominis*, produce antimicrobial peptides capable of inhibiting *S. aureus* growth. Clinical studies have shown that applying these beneficial *Staphylococcus* strains to AD-affected skin can reduce *S. aureus* abundance. Additionally, transplantation of *Roseomonas mucosa* strains derived from healthy individuals has been associated with improved skin symptoms in AD patients. Another promising approach involves targeting the agr quorum-sensing system of *S. aureus*, which regulates the production of virulence factors, including the g-toxin. In preclinical models, inhibiting this system has demonstrated therapeutic potential.[141,142]

The presence of *S. aureus* on AD-affected skin has also been linked to elevated levels of Th2-associated biomarkers, highlighting a relationship between microbial dysbiosis and immune dysregulation. Dupilumab, an IL-4 receptor antagonist, disrupts Th2 signaling and may indirectly prevent *S. aureus* colonization by altering bacterial cell wall proteins. Emerging systemic biologic therapies are expected to not only modulate immune responses but also influence *S. aureus* colonization patterns. Research into the pathogenicity of *S. aureus* strains isolated from AD patients has revealed differences in their cell wall proteins and secreted virulence factors compared to standard *S. aureus* strains. These microbial components may serve as novel therapeutic targets. However, key questions remain, including how Th2-driven inflammation affects the regulation of *S. aureus* virulence factors and how existing treatments, such as topical corticosteroids and biologics, influence bacterial pathogenicity. [141,142]

The role of *S. aureus* in AD extends beyond allergic hypersensitivity, with implications for skin immunity. Given this complexity, microbiome-based immunotherapies represent a promising avenue for AD treatment. Unlike conventional corticosteroids, these therapies aim to restore microbial homeostasis and modulate immune responses through distinct mechanisms. As research advances, microbiome-targeted interventions may provide a foundation for novel therapeutic strategies to improve long-term AD management. [141,142]

The application of machine learning has led to the development of a highly accurate predictive model, demonstrating that residual methotrexate concentrations following ex vivo incubation with pre-treatment samples from patients with newly diagnosed RA correlate with the extent of future clinical response. This finding implies a direct influence of the gut microbiome on methotrexate absorption and therapeutic efficacy. Collectively, these insights mark an initial step toward leveraging the gut microbiome as a tool for predicting responses to oral methotrexate in early RA and potentially as a target for microbiome-based interventions in autoimmune and rheumatic diseases. Ongoing research is exploring whether the gut microbiome also modulates the response to parenterally administered methotrexate and biologic therapies, as well as whether microbiome-driven predictive models can be extended to other oral disease-modifying antirheumatic drugs (DMARDs), such as JAK inhibitors.[144]

Expanding knowledge in pharmacomicrobiomics holds significant translational value in understanding both drug effectiveness and adverse reactions to commonly used rheumatologic treatments. Given the substantial and unpredictable variability in individual responses to methotrexate, sulfasalazine, and other synthetic or biologic DMARDs, precision medicine approaches that incorporate gut microbiome characteristics could improve the rational selection and use of these therapies (Figure 4.6).[144]

From a diagnostic perspective, pharmacomicrobiomics may be applied in rheumatology through the identification of microbial species, genes, transcripts, or proteins that influence drug metabolism, molecular transport, or immune modulation. Such insights could enable both clinicians and patients to make informed therapeutic decisions based on the gut microbiome profile prior to treatment initiation. This approach could guide the selection of drugs more likely to achieve favorable outcomes while also opening new avenues for microbiome-modulating strategies—whether used sequentially or as adjunct therapies—to optimize drug bioavailability and symptom relief.[144]

As seen in oncology, where baseline gut microbiota has been used to predict treatment response and colitis risk in checkpoint inhibitor trials, as well as in fecal microbiota transplantation (FMT) for colitis management, similar strategies are now being applied to inflammatory arthritis along with ankylosing spondylitis adapting also a Mediterranean diet. One example is the FLORA study, a randomized, placebo-controlled clinical trial investigating FMT in patients with psoriatic arthritis who have shown inadequate responses to methotrexate. This study showed that increased intestinal permeability, along with distinct fecal and plasma metabolomic profiles, was observed in patients with active peripheral PsA and was linked to the primary clinical outcome (non-responders vs. responders) in the first randomized trial assessing the safety and efficacy of single-donor FMT. These findings further support the connection between PsA and the complex interactions between the host and microbiota, highlighting the potential for therapeutic strategies focused on restoring microbiome balance and/or targeting specific microbial metabolites and host-microbiota pathways. However, additional randomized controlled trials are necessary to fully understand the clinical significance of these results. [144,147,148]

While these pioneering studies will provide valuable insights, alternative microbiome-targeting interventions are also being explored. Less invasive methods, such as prebiotic and probiotic supplementation, may offer comparable benefits without the logistical challenges and potential risks associated with FMT, including procedural complications, limited clinical practicality, and the possibility of pathogen transmission. [144,145]

Additionally, emerging technologies such as organ-on-a-chip models (e.g., gut-on-a-chip) and bacterial culturomics are being utilized to enhance our understanding of pharmacomicrobiomics. These platforms replicate the intestinal microenvironment, allowing for the study of host-microbe interactions under controlled conditions. By incubating drugs of interest in these systems, researchers can assess their impact on bacterial growth and metabolism while uncovering the mechanisms by which bacteria modify drug activity.[144,145]

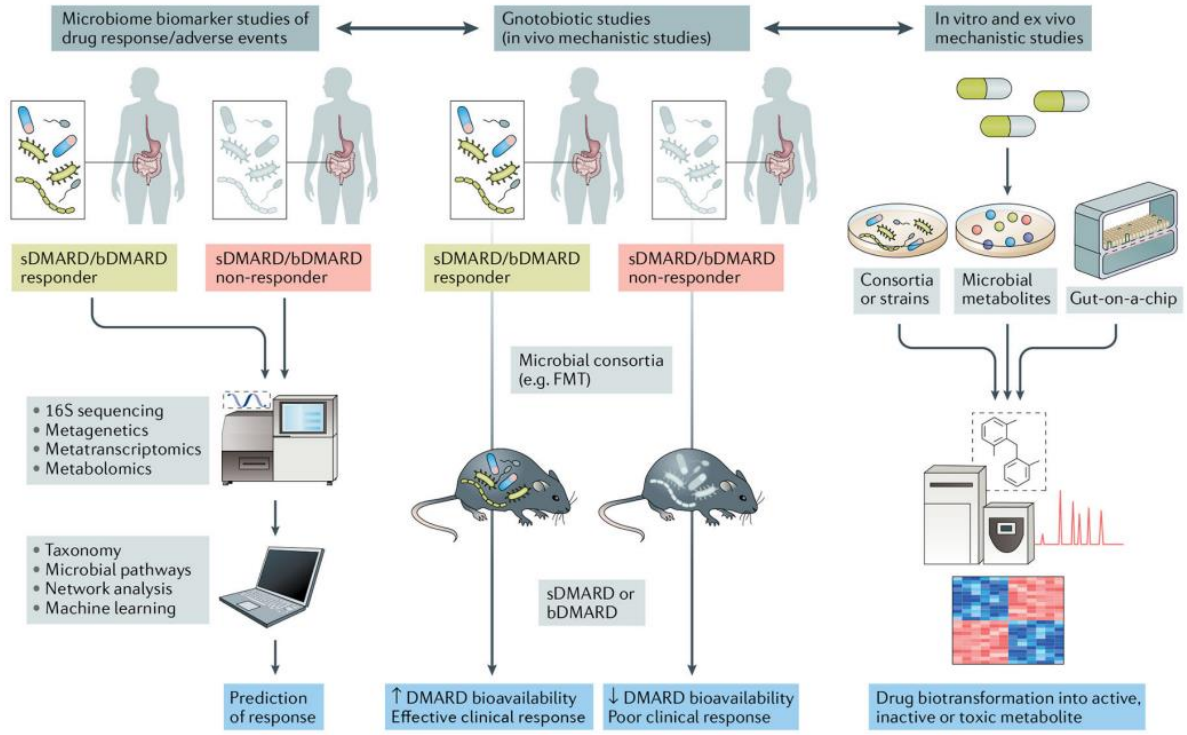

Figure 4.5 Pharmacomicrobiomic studies in rheumatic diseases [144]

4.1.8.2 Challenges and future directions for microbiome research in immunology

Investigating the gut microbiome presents distinct challenges due to its complex nature and the intricate interactions between microbial populations and host health. A primary difficulty is the extensive diversity and variability in the gut microbiome among individuals, influenced by factors such as diet, genetics, age, and environmental conditions. This variability complicates the identification of universal microbiome profiles linked to either health or disease. To address this, large-scale studies are required to capture this diversity and understand its relevance to human health. Furthermore, establishing causal relationships between specific microbial communities and health outcomes is challenging due to this variability, necessitating advanced analytical techniques. A major hurdle in microbiome research is distinguishing correlation from causation. While many studies have revealed associations between certain microbial patterns and health conditions, demonstrating that these microbes directly contribute to or cause these conditions is more complex. This is partly because animal models, while valuable, cannot fully replicate the complexity and diversity of the human microbiome. This limitation underscores the need for innovative experimental designs that better reflect human microbial ecosystems.[144-146]

In addition, technical and methodological challenges persist in microbiome research. High-throughput sequencing techniques, such as 16S rRNA gene sequencing and shotgun metagenomics, have significantly advanced our understanding of the gut microbiome. However, these methods have their drawbacks, including biases during DNA extraction and amplification, as well as difficulties in interpreting the vast data generated. Enhancing bioinformatics tools and databases is crucial for improving the accuracy of microbial species identification and functional characterization. While shotgun metagenomics provides a more

comprehensive overview of species, it tends to detect fewer taxa at the family and genus levels compared to 16S rRNA sequencing, which excels in detecting genus-level diversity. Both methods have their respective advantages, and the choice between them depends on the specific research objectives and available resources. [144-146]

Translating microbiome research into clinical practice also faces challenges. While microbiome-based interventions hold promise for disease prevention and treatment, the development of such therapies is complicated by factors like individual microbiome variability, the potential for unintended effects, and the necessity for personalized treatments. A multidisciplinary approach, integrating microbiology, immunology, genetics, and medicine, is essential to overcome these hurdles and ensure ethical and regulatory considerations are addressed. Bridging the gap between research and clinical application will be critical for maximizing the potential of microbiome-based therapies in healthcare. [144-146]

The safety and efficacy of interventions such as FMT or probiotic supplementation also pose significant challenges. While these approaches show promise, they require rigorous clinical trials to assess their therapeutic potential and possible side effects in humans. Furthermore, the regulatory landscape for such treatments remains uncertain, adding complexity to their clinical application. The interaction between the gut microbiome and pharmaceuticals is another layer of complexity. Certain drugs, like non-steroidal anti-inflammatory drugs commonly used in orthopaedic care, can alter the gut microbiome. Understanding these interactions is key to developing treatment plans that integrate both the benefits of medications and their microbiome-mediated effects. [144-146]

As research on the gut microbiome's impact on inflammatory diseases improvements progresses, several promising future directions are emerging. One major opportunity is the

development of precision microbiome therapies tailored to individual patient profiles. By identifying microbiome configurations associated with favorable clinical outcomes, researchers could create targeted probiotic or prebiotic treatments to maintain a healthy microbiome balance. Such approaches may help reduce the risk of diseases like inflammatory arthritis. The integration of machine learning and artificial intelligence into microbiome data analysis could lead to the discovery of new microbial markers for early disease detection and risk assessment, enabling more proactive management and potentially altering disease trajectories. The therapeutic potential of FMT in inflammatory disorders also warrants exploration. While FMT has shown effectiveness in treating conditions like *Clostridioides difficile* infections, its potential in modulating the gut microbiome for inflammatory improvement outcomes remains an exciting area for investigation. Studying the safety, efficacy, and long-term effects of FMT in conditions such as rheumatoid arthritis, atopic dermatitis could open new therapeutic pathways. [144-146]

Understanding the gut-skin-bone axis is another promising avenue for advancing inflammatory improvements. Recent research suggests a complex relationship between the gut microbiome, skin microbiota, and bone health, indicating that systemic interactions may play a significant role in musculoskeletal diseases. Future studies investigating these interconnected pathways may uncover multifaceted treatment strategies involving diet, lifestyle, and microbiome modulation to support bone health. [144-146]

Finally, the ethical, legal, and social implications of using gut microbiome data in clinical practice must be carefully considered. As microbiome-based diagnostics and treatments move closer to clinical application, concerns related to privacy, consent, and the impact of microbiome data on insurance and employment must be addressed. Ensuring that microbiome

advancements are incorporated into healthcare in an ethical and equitable manner is vital. [144-146]

In conclusion, the growing field of gut microbiome research offers tremendous potential to transform inflammatory immune diseases. By improving our understanding of the complex relationships between the gut, immune system, and bones, microbiome-based therapies could revolutionize the personalized care of IMIDs. However, to realize this potential, challenges such as improving clinical translation, standardizing methodologies, and addressing ethical considerations must be met with a collaborative, multidisciplinary effort. [144-146]

Chapter 5: DISCUSSION

The findings of this dissertation highlight the intricate relationship between the microbiome and immune-mediated inflammatory diseases (IMIDs), reinforcing the notion that microbial dysbiosis plays a pivotal role in the pathogenesis and progression of these disorders. The microbiome's impact on immune regulation, inflammatory processes, and systemic homeostasis underscores the need for a more comprehensive understanding of host-microbe interactions. The growing body of evidence linking gut, skin, and other site-specific microbiomes to IMIDs suggests that the interplay between microbial communities and host immunity is a key determinant in disease development and potential therapeutic interventions. Given that the immune system is heavily shaped by microbial interactions, the microbiome is increasingly recognized as a key player in immune-mediated diseases, influencing disease onset, progression, and therapeutic response. [149-160]

Despite significant advances, microbiome research faces several challenges. One major limitation is the variability of microbiome composition across individuals, influenced by genetic, environmental, and lifestyle factors. This heterogeneity makes it difficult to establish universal microbiome signatures for specific IMIDs. Furthermore, standardization of microbiome research methodologies remains a hurdle, as differences in sequencing technologies, analytical pipelines, and sample collection methods can lead to inconsistencies in study outcomes. Another challenge is the causality dilemma—whether microbiome alterations are a cause or consequence of immune dysregulation. While associations between dysbiosis and IMIDs have been well-documented, establishing causative mechanisms requires longitudinal studies and functional analyses. Confounding factors such as diet, medication use (e.g.,

antibiotics, immunosuppressants), and lifestyle choices further complicate the interpretation of microbiome contributions to immune-mediated diseases. [149-160]

Recent research has identified key microbial taxa associated with different IMIDs, highlighting their potential as predictive biomarkers and therapeutic targets. In AS, *Klebsiella pneumoniae* has been implicated in immune dysregulation via molecular mimicry, particularly in genetically susceptible individuals carrying HLA-B27. AD is characterized by an overrepresentation of *Staphylococcus aureus* on the skin and a reduced abundance of beneficial *Bifidobacterium* and *Lactobacillus* species in the gut, contributing to skin barrier dysfunction and immune hyperreactivity. In RA, *Prevotella copri* is often overrepresented in the gut, potentially inducing pro-inflammatory responses through Th17 activation, whereas a reduced presence of *Faecalibacterium prausnitzii*—a key butyrate-producing bacterium—is associated with disease severity. Psoriasis and PsA are linked to decreased levels of *Akkermansia muciniphila* and Bacteroides, both crucial for maintaining gut barrier integrity, which may exacerbate systemic inflammation. IBD, encompassing Crohn's disease and ulcerative colitis, is associated with a depletion of *Faecalibacterium prausnitzii* and an increase in *Escherichia coli*, particularly *adherent-invasive E. coli*, which contributes to gut epithelial damage and chronic inflammation. Multiple Sclerosis (MS) patients exhibit gut microbiota alterations with an increased abundance of *Akkermansia muciniphila* and *Methanobrevibacter*, while beneficial taxa like *Butyricicoccus* and *Parabacteroides* are reduced, suggesting a link between gut dysbiosis and neuroinflammation via the gut-brain axis. [152-170]

Similar microbiome alterations have been consistently reported in IBD, especially Crohn's disease, highlighting a common thread across IMIDs. Given the shared genetic predispositions and overlapping phenotypic features of spondyloarthritis, it is plausible that dysbiosis represents a common trait across IBD, AS, and PsA. This raises important questions regarding

whether microbial imbalances are a primary driver of systemic inflammation or merely a consequence of the disease state. Addressing this question will require advanced longitudinal studies and functional microbiome analyses to delineate causality and potential therapeutic targets. [152-170]

These microbial taxa provide crucial insights into the pathophysiology of IMIDs and may serve as pillars for precision medicine, guiding the development of personalized interventions. The integration of artificial intelligence (AI) and precision medicine is expected to revolutionize microbiome research and clinical applications. AI-driven analytics can process complex microbiome datasets, enabling disease prediction, patient stratification, and biomarker identification with higher accuracy. Furthermore, precision medicine approaches that incorporate microbiome profiling alongside genomic and metabolomic data could pave the way for personalized therapies, such as microbiome-modulating interventions tailored to individual microbial compositions. Emerging strategies such as CRISPR-based bacterial modification, synthetic microbial communities, and targeted probiotic therapies hold significant promise in restoring microbial balance and mitigating immune dysregulation in IMIDs. [149-170]

Future perspectives in personalized microbiome therapies are promising, as advancements in multi-omics technologies facilitate the development of targeted interventions. The use of next-generation probiotics, engineered bacterial strains, and postbiotics offers novel therapeutic avenues to reshape the microbiome and restore immune homeostasis. Additionally, fecal microbiota transplantation continues to gain traction as a potential intervention for rebalancing dysbiotic microbial communities in IMIDs. The incorporation of AI-driven predictive models can further enhance treatment efficacy by identifying patient-specific microbial signatures that correlate with therapeutic response, ultimately optimizing treatment strategies for IMIDs. [149-170]

Chapter 6: CONCLUSION

This dissertation provides a comprehensive review of the association between the microbiome and immune-mediated inflammatory diseases, emphasizing the role of microbial dysbiosis in disease onset and progression. The findings consolidate existing evidence that the gut, skin, and other microbiomes are not merely passive inhabitants but active players in immune regulation and inflammation. Although microbiome research has made remarkable strides, significant challenges persist, particularly in understanding the causal mechanisms of dysbiosis, standardizing research methodologies, and addressing inter-individual variability. The integration of AI and precision medicine holds great promise for overcoming these barriers, enabling the development of personalized microbiome-based interventions**.** Looking ahead, a deeper understanding of host-microbiome interactions will be essential for translating microbiome research into targeted therapies for IMIDs. By leveraging advances in microbiome sequencing, computational biology, and systems immunology, future research can pave the way **o**r microbiome-driven precision medicine, ultimately improving patient outcomes in immune-mediated diseases.

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