anatomia

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Review

The Microbiota Is Not an Organ: Introducing the Muco-Microbiotic Layer as a Novel Morphofunctional Structure

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Abstract: In this paper, we want to refute the notion that the microbiota should be considered an organ, given that an organ comprises tissue of similar or different embryological origin, while the microbiota is a pool of different microbial species originating individually from single replications and not from a common ancestral cellular element. Hence, we would like to propose a new morphological interpretation of its nature, based on the comprehensive context in which these microbes live: a muco-microbiotic layer of hollow organs, such as the airways and the bowel. The above concept should represent not only a new terminological annotation but also a more accurate portrayal of the physiology and pathophysiology of these organs. Indeed, a better understanding of the biological nature of this part of the human body can help scientists develop more specific experimental protocols, potentially leading to the establishment of better therapeutic strategies.

Keywords: airways; bowel; respiratory system; digestive system; microbiota; muco-microbiotic layer; cell differentiation; tissue homeostasis; organ remodeling; nanovesicles

Citation: Fucarino, A.; Burgio, S.; Paladino, L.; Caruso Bavisotto, C.; Pitruzzella, A.; Bucchieri, F.; Cappello, F. The Microbiota Is Not an Organ: Introducing the Muco-Microbiotic Layer as a Novel Morphofunctional Structure. *Anatomia* **2022**, *1*, 186–203. https://doi.org/10.3390/anatomia1020019

Academic Editors: Gianfranco Natale and Francesco Fornai

Received: 20 July 2022
Accepted: 8 November 2022
Published: 14 November 2022

1. Brief Introduction to the History and Terminology Related to the Term "Microbiota" and about Its Definition in Scientific Literature

Although it is often claimed that the terms "microbiota" and "microbiome" were coined by the Nobel laureate microbiologist Joshua Lederberg in 2001, Susan L. Prescott [1] clarified that this is not correct. Lederberg did not come up with these terms, simply because they are both basic microbiology terms that had already been in common use for many decades before becoming widely used. The terms are often used synonymously by the scientific community; however, they are two separate entities. In fact, "microbiota" describes specific microbe populations that are found within a specific environment (i.e., a hollow organ) comprising bacteria, fungi, archaea, protists, helminths and viruses that symbiotically inhabit the host [2], while "microbiome" refers to the sum of all microbes and their genes. In this paper, we discuss only the microbiota.

Furthermore, a number of researchers would describe the microbiota as an "additional organ" of our body [3], using such terms as "organ-like collection of microbes," "microbial organ," "microbial system," or "metabolic organ" [4], all of which are devoid of scientific significance. At the same time, novel conceptions have been formulated to address its biological and ontological significance, such as "holobiont" and "hologenome" [5,6], although these proposals are still under debate.

In this brief paper, we present our vision of the microbiota from a strictly morpho-functional point of view. In particular, we illustrate the microbiota in a histological context, focusing our attention on its significance in the physiology and pathophysiology of two anatomical districts—the airways and the intestine.

2. Microscopic Anatomy of Human Airways and Bowel: Similarities and Differences

We would like to include just a few lines to briefly recapitulate some basic anatomic and histologic knowledge that can be useful for those readers of this manuscript who may not be experts in this field. For further details, any good anatomy and histology textbook can be consulted.

In human anatomy, organs are subdivided into two categories: hollow and parenchymatous. Hollow organs, in structural terms, have a wall composed of several layers. These layers are formed by tissue, which in turn are formed by cells, each one having a functional specialization deriving from differentiation.

Human anatomy aims to describe cell differentiation, tissue homeostasis and organ remodeling during the life span of an individual (from zygote formation to the death of the organism). Any alteration in cell differentiation (for any reason, intrinsic or extrinsic) leads to tissue disruption and organ dysfunction, in turn determining the onset of a disease. Therefore, diagnostic approaches, as well as the development and perfection of treatment approaches, are reliant on sufficiently understanding these processes.

Airways are part of the respiratory system, together with the lungs. They are divided into upper and lower ones. The former includes the nasal and paranasal cavities and pharynx; the latter includes the larynx, trachea, and bronchi. Both the upper and the lower airways are hollow structures. The walls of the lower airways are made up of three layers: mucosa, submucosa (or fibromuscular cartilaginous layer) and adventitia (Figure 1A). The mucosa is the most internal one, comprising a lining of pseudostratified ciliated mucous epithelium lying on a connective lamina propria hosting mucous glands, as well as hematic and lymphatic vessels, and populated by fibroblasts and immune cells. More recently, the concept of an epithelial–mesenchymal trophic unit (EMTU) has been introduced to better explain the close relationship between the epithelium and the lamina propria in all homeostatic processes in these tissue types [7,8].

Evans et al. theorized the idea of the EMTU at the beginning of the 2000s. According to their hypothesis, the epithelial layer and underlying mesenchymal cells communicate to control and regulate responses to external environmental stimuli [9]. This communication network plays a key role in many different aspects of airway homeostasis. Both the internal and external communication of the EMTU hinges on cytokines and signaling molecules released by both cell populations [10,11]. Processes like embryogenesis and airway differentiation use EMTU signaling [12,13], and a dysregulation of these pathways can lead to the development of various pathologies, such as chronic obstructive pulmonary disease (COPD), asthma, bronchiolitis obliterans syndrome, and idiopathic pulmonary fibrosis [14–17].

The bowel is part of the digestive system and comprises the small and large bowel. Both are hollow structures and have a wall divided into four layers (Figure 1B): mucosa, submucosa, muscularis propria, and adventitia (replaced by the serosa, i.e., the peritoneum, in some areas). The bowel mucosa is made of three overlapped tissue types: the lining of monolayered columnar epithelium composed mainly of adsorbent and mucous cells (but containing also other cytotypes; see specialized textbooks for details); the lamina propria, containing, among others, glands, hematic and lymphatic vessels, fibroblasts, and immune cells; and the muscularis mucosae, representing the border between the connective tissue of the lamina propria and that of the submucosa.

The bowel and the airways can be considered two remarkably similar structures from the following points of view. Both are in fact covered with a layer of mucus that is produced by the mucous cells and glands of the epithelium and houses the microbiota, constituted of about 100 trillion bacterial, archaeal, viral, and eukaryotic domains, encompassing more than 1000 different species [18–23]. This mucus also houses extracellular vesicles that are very useful tools of trafficking between human cells and the microbiota, actively involved in maintaining their homeostasis.

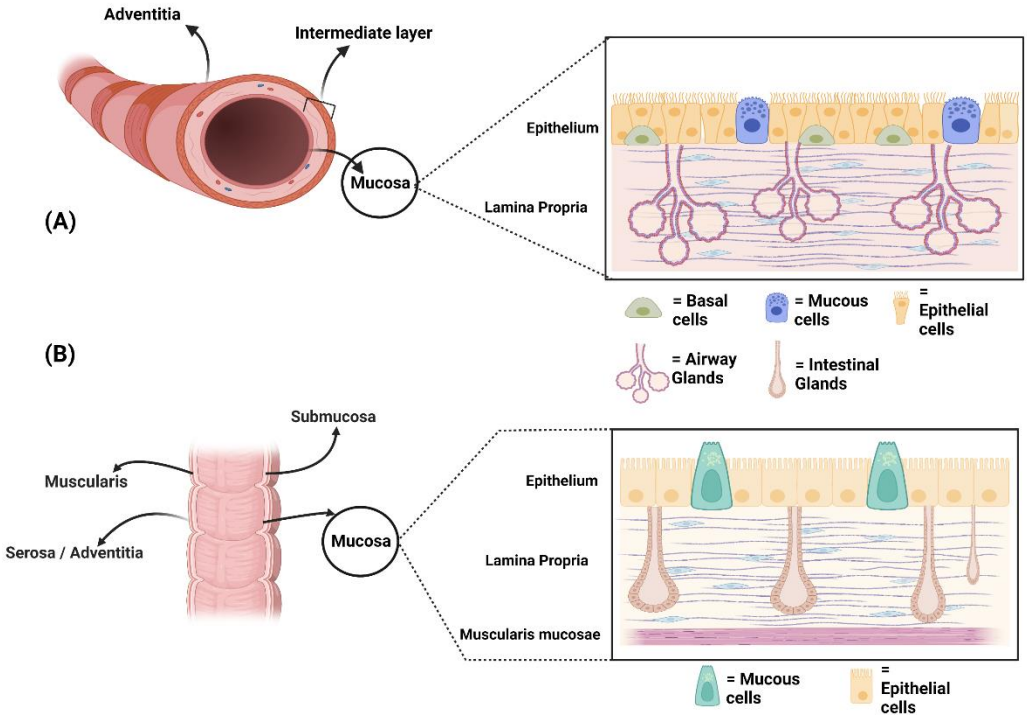

Figure 1. Components of the airway (**A**) and bowel (**B**) mucosa, as classically described in anatomical and histological literature. In brief, both organs have a monolayer of epithelial cells with an underlying lamina propria, in which glands can be found. We can appreciate morphological differences between airway and intestinal glands: airway glands have a tubuloacinar structure, single terminal ducts and branched secretory tubules that end in serous acini; intestinal glands generally present unbranched tubules that end in a single acinus. The muscularis mucosa is present only in the bowel, separating the connective tissue of the lamina propria from that of the submucosa. See the text for further details.

The microbiota presents great differences in relation to age, ethnicity, eating habits, seasonal changes, immune status, and other physiological variables, both in the airways [21–24] and in the bowel [25–30]. These findings have prompted several studies on its role during the pathogenesis of various diseases of the respiratory and the digestive systems (including inflammatory and tumoral ones), the systemic complications caused by such disorders, and last but not least, the therapeutic potential of altering the microbiota with the use of prebiotics and probiotic or microbiotic transplants [31–36]. Here we want to focus on and compare the microbiota of the airways and the bowel, in the context of microenvironment.

3. The "Ghost" Layer

The classic description of airway and bowel structure made in the previous paragraph does not take into account the fact that in living subjects, the innermost lining of the intestinal lumen is not the mucous layer, but mainly consists of a mix of mucus, microbiota, and myriad soluble factors in which nanovesicles are dispersed. For this reason, we propose using the term "muco-microbiotic (MuMi) layer" to describe the innermost lining of both airway and bowel walls in living subjects (Figure 2).

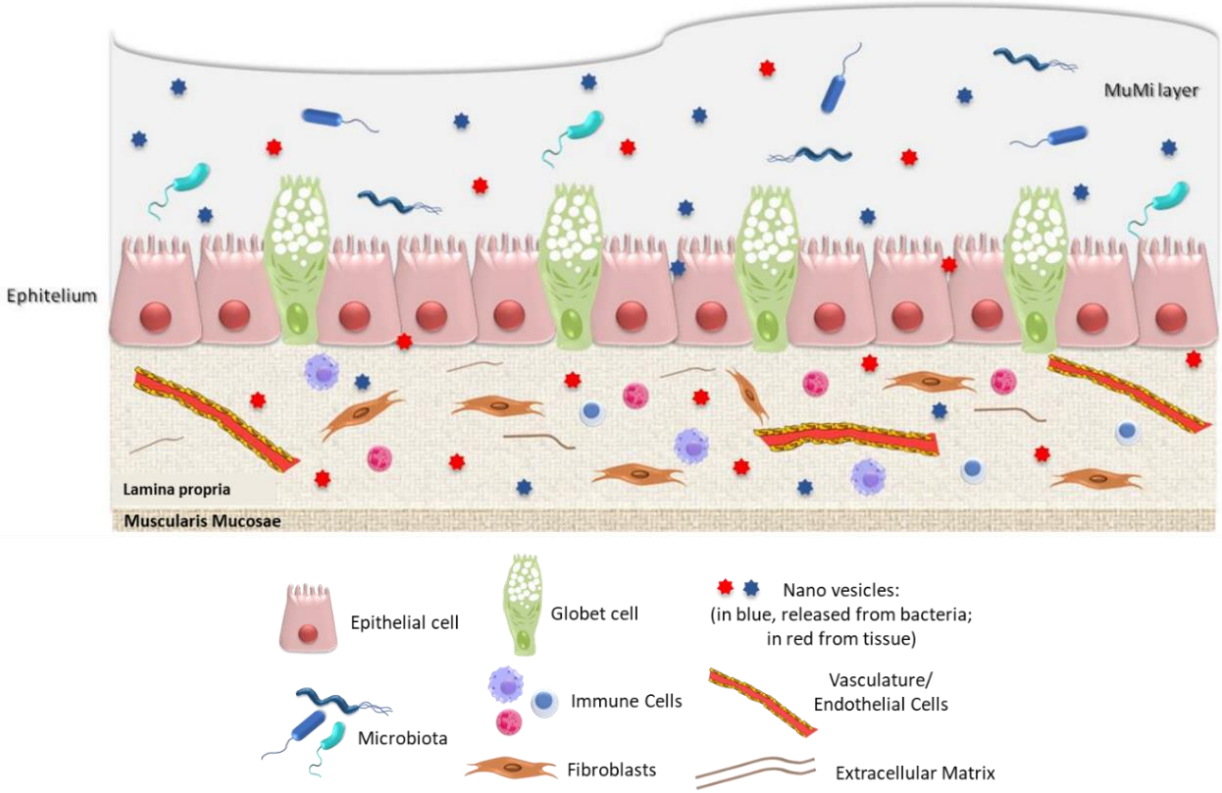

Figure 2. Main components of the muco-microbiotic layer (i.e., mucus, microbiota, and nanovesicles) in the bowel mucosae and its relationships with epithelial elements and connective cells of lamina propria. See the text for further details.

Enterocytes, the cells responsible for the absorption of nutrients, and goblet cells, the elements secreting the mucus that covers the intestinal wall, are the most common cytotypes that make up the bowel epithelium. The MuMi layer represents the first line of defense against various types of stress, such as ingested material, hydrochloric acid, proteolytic enzymes, etc., that the bowel is exposed to. The mucus, synthesized and secreted by goblet cells, is colorless, since it is mainly composed of water (up to 95%), lipids and small proteins. Its mechanical and viscoelastic properties are guaranteed by the presence of high-molecular-weight glycoproteins, identified as mucins [37,38]. The mucus is denser at the epithelial level and less so closer to the lumen, being also exposed to bacterial proteolytic activity. In fact, different microbial species of the normal microbiota are present in the MuMi layer. This allows the formation of a layer innermost to the intestinal lumen, that we call for the first time the "MuMi layer", produced by the union of the mucus layer, microorganisms, and soluble factors, including extracellular vesicles (EVs) [39]. EVs represent the main dialogue tool between the microbiota and human cells of the mucosal layer, including not only epithelial cells but also fibroblasts and immune elements distributed in the lamina propria.

The MuMi layer is not visible under routine histologic examination because it is lost during the processing of biopsies for microscopic observation, due to the solubility of the mucus in alcoholic solutions. Consequently, this internal lining has been systematically missed by histological studies and generally ignored for a long time, despite the fact that in our opinion, it has a key role in airway and intestinal physiology and pathophysiology (as discussed below).

It is also important to remember that both the airway and intestinal tracts establish multiple relationships with other anatomical districts through nanovesicular trafficking. In fact, exosomes and outer membrane vesicles—produced, respectively, by human and bacterial cells—are able to reach, through the bloodstream, virtually any anatomical district, including some "protected sanctuaries," such as the brain, the testicles, and the

thymus [35,36]. Due to its wide extension all along the hollow organs, this layer releases a great quantity of nanovesicles of both bacterial and human origin, in addition to other soluble factors with autocrine- and paracrine-like effects capable of reaching the general circulation and producing systemic effects.

In this context, the MuMi layer should be seen as a morphofunctional layer more than merely an anatomical one, because it is not a derivative of any embryonic structure, unlike the remaining layers of the GI tract or the airway. However, since it represents the symbiotic relationship between the microbiota and the host cells, we cannot ignore the existence of this layer, for reasons better clarified in the following paragraphs.

4. Extracellular Vesicles: Physiology and Pathophysiology

Extracellular vesicles (EVs) are nanosized lipid membrane vesicles released by virtually all human cells involved in many cellular processes, both in physiological and pathological conditions, such as cell differentiation and tissue homeostasis [40–42].

EVs are classified into different subtypes based on their biophysical features, such as size, density, and biochemical composition. According to this classification, it is possible to describe "small EVs" with ranges between 50 nm and 200 nm and "medium/large EVs" sized >200 nm. However, this classification frequently refers to the traditional nomenclature based on EV dimension and mechanism of biogenesis: (a) exosomes of 20–200 nm of diameter that are formed within the endosomal network and released upon the fusion of multivesicular bodies with the plasma membrane; (b) microvesicles with a diameter between 200 nm 1000 nm (also called microparticles and ectosomes) that are produced by outward budding and fission of the plasma membrane; and (c) apoptotic bodies sized >1000 nm that are released as blebs of cells during apoptosis [43,44].

EVs are present in many different biofluids, such as blood, lymph, breast milk, saliva, urine, and cerebrospinal fluid, carrying into the extracellular compartment as well as into distant districts, lipids, nucleic acids and proteins, which represents a way of long-distance communication and horizontal transfer of genetic material. EV content varies according to the type of cell that released it, thus providing information on its status [40,45]. Furthermore, it has been shown that EV-carried molecules modulate and reprogram recipient cells, mediating signal transduction between cells and influencing their behavior [46].

In addition to participating in some fundamental physiological processes, EVs have also been implicated in disease development, favoring, for instance, tumor cell proliferation, angiogenesis, extracellular matrix degradation, and immune response deregulation [47]. Therefore, investigation of EVs is useful to highlight mechanisms that may determine health or disease. Furthermore, as EVs carry genetic material and proteins that may affect different signaling pathways in the target cells and organs, and since they are commonly found in biological fluids and tissue, they can be used as noninvasive and easily accessible disease biomarkers [48–53]. As stated above, in different diseases, cells can produce EVs with alterations in number and molecular content compared to physiological conditions, and accumulating evidence demonstrates their role in cancer, in which the application of EVs may be helpful for early diagnostics and the identification of new therapeutic targets [52].

In the airways, secreted EVs are involved in lung homeostasis and pathologies, such as inflammatory disease and lung cancer [54], and have been reported in plasma and secretions from airways, such as saliva [55], induced sputum [56] or bronchoalveolar lavage fluid (BALF) [57]. Among the cells of the pulmonary microenvironment that secrete EVs, bronchial epithelial cells and alveolar macrophages are the major sources, and it has been demonstrated that their molecular content has some immunomodulatory effects and defense functions of the airway in lung inflammatory and chronic diseases, such as asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and lung cancer [54,58].

Furthermore, in the bowel tract, secreted EVs act as communicators between intestinal cells and as long-distance mediators involved in maintaining immune homeostasis. EVs produced by intestinal layers, including the MuMi one, have different effects, such as playing a role in the repair mechanisms of epithelium integrity [59] and in the activation of immunological pathways [60,61]. At the same time, they can become a disease biomarker when isolated from circulation or other biological fluids [53]. Moreover, EVs released by intestinal layers seem to be involved in the defense mechanism against pathogenic microbes [60], and consequently have important roles in the cross talk between the host body and resident microorganisms [62].

As a matter of fact, EVs are naturally produced by both Gram-negative and Gram-positive bacteria harbored in the airways and the bowel. They are called outer membrane vesicles (OMVs) or membrane vesicles (MVs), respectively [63–65]. These particles are ubiquitously produced by blebbing of the outer membrane or following bacterial lysis, and contain a variety of molecules, such as proteins, RNAs, DNAs, lipids and enzymes, whose loading mechanism is not yet fully understood [63–65].

EVs produced by the airway microbiota have important roles in activating immune responses, such as *Staphylococcus aureus*- and *Pseudomonas aeruginosa*-derived EVs, which may induce neutrophilic pulmonary inflammation [66,67]. Initially, it was believed that intestinal bacterial EVs may have a pathogenic role, carrying toxins and virulence factors favorable to establishment of infection [68]. Subsequently, it was observed that microbiota-derived EVs can also initiate host immune responses and cross the intact intestinal epithelial barrier to reach distant tissue [69]. In fact, like the exosomes, OMVs and MVs play an important role in communication with neighboring bacteria and the environment, being able to travel long distances, transporting their contents throughout the body. This could lead us to hypothesize that OMVs also act elsewhere, other than the airway and bowel lumen, amplifying the role of the microbiota [70].

The following paragraphs analyze the composition of both the mucus and the microbiota in the airways and the bowel in better detail to add important information for a better understanding of the role of the MuMi layer in human physiology and pathophysiology.

5. Airway Composition

The respiratory tract represents a large surface area that interacts with the external environment. As a physical barrier, the airway epithelium and the bronchial mucus are classically considered essential for the protection of both the airways and the lungs from any external etiological agents, including microbes and allergens [71]. Airway mucus, produced by the submucosal glands, is a complex dilute aqueous solution resulting from the sum of proteins, glycoconjugates, and lipids, containing electrolytes, enzymes, anti-enzymes, oxidants and antioxidants [72]. Under normal conditions, mucus is scarce in quantity but still present, and forms a thin layer that homogeneously covers the surface of the airways, from the upper airways to the bronchi. In fact, the first, most fundamental function of this thin mucous layer is to ensure the maintenance of appropriate humidity and temperature levels of the incoming air flowing through the respiratory tree. This is also important to ensure a favorable environment for the colonization of bacteria, viruses and fungi that compose the pulmonary microbiota.

In fact, the respiratory tract is inhabited by niche-specific communities of bacteria acting as gatekeepers that provide resistance to colonization by respiratory pathogens. A variety of different microbial species colonize the airways right after birth, depending on the type of delivery. Significant changes occur during the first year of life, driven by both immune system maturation and diet [73]. Thus, the microbial community in infants and children gradually transforms into the adult respiratory tract microbiome, becoming less dense but more diverse [74].

The lower respiratory tract is one of the least-populated surfaces of the human body in terms of the microbiome, with approximately 10–100 bacteria per 1000 human cells [75]. In particular, airways exhibit a gradient of bacteria burden that is relatively high in the upper tract and substantially diminished in the lower one (Table 1) [76]. The mucous surfaces of the upper respiratory system are colonized by a wide range of bacteria belonging to the genera Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria. While it was previously assumed that the lower respiratory tract was sterile, except during infection, recent studies have shown the presence of bacteria, such as Firmicutes, in the lungs of healthy individuals [16,74,77–79]. The different distribution of bacteria genera is probably ascribable to the different characteristics of the airway's tracts. In the upper section of the respiratory tract, the inhaled air is heated, humidified, and filtered. These processes affect both bacteria from the external environment and those that reside permanently in our body. The selection of genera leads to the formation of a specific symbiotic microbiome for the different portions of the airways [76].

Generally, differences in temperature, pH, mucus secretion, and relative oxygen concentration regulate bacterial colonization in the respiratory tract. Several environmental factors, such as age, weight, and tobacco smoke exposure, can damage the epithelium and facilitate microbial entry into the host, contributing to dysbiosis [80]. Similarly to the gut, airway dysbiosis is associated with local inflammation and exacerbation of many pulmonary disorders, such as COPD or asthma [81]. Clinical features of inflammation include increased vascular permeability, facilitating the release of immune cells and mucus secretion [82,83].

Moreover, the airway microbiota, along with the mucus, plays a fundamental role in the barrier function of the airway epithelium, maintaining a stable homeostasis [71], and a dialogue is likely to develop between specific bacterial communities and the host in a dynamic way. In particular, the loss of barrier function can affect the composition of the respiratory microbiota, allowing the entry of pathogens and other particles. As mentioned before, the mucus maintains an efficient airway environment, acting as a physical and biological barrier and ensuring the humidification of the air that passes through the trachea and the large bronchi.

Numerous experimental studies have shown that in the absence of mucus, the respiratory tract changes profoundly, often causing substantial damage or alterations to the anatomical structures of the lung [84]. It is well known, for example, that the absence of mucus in the airways can cause an irritative inflammatory reaction [84]. It also constitutes an important filter and dilution tool: many types of gaseous substances that are irritating or toxic to the respiratory tract are subjected to a process of filtration and dilution by the mucous surface of the airways. It is also used to remove particles, dust and biological agents.

On the other hand, emerging experiments have highlighted a crucial cross talk between the intestinal microbiota and the respiratory one, called the "gut–lung axis," highlighting the possible involvement of microaspiration of intestinal microbes in the development of the airway microbiota [75,84]. In fact, a change in the constituents of the gut microbiome could alter immune responses and homeostasis in the airways. Various factors, such as diet, have been shown to not only impact the composition of the intestinal microbiota but also the respiratory one, indicating that the intestinal and respiratory compartments are closely interconnected, and that changes at one of the two sites could impact the other [71,75,84].

Short-chain fatty acids (SCFAs), the metabolites produced by the gut microbiome in the intestinal microenvironment, have been shown to reach other organs and to play a role in respiratory diseases, such as asthma [75]. In fact, several studies have shown that a diet rich in fiber causes an increase in circulating SCFA levels produced by the fermentation of anaerobic bacteria. SCFAs promote leukocyte recruitment and immune regulation within the inflammatory process, preventing allergy- and asthma-related lower airway inflammation [16,85,86].

However, despite several studies demonstrating a crucial and beneficial role of the microbiota, the usefulness of probiotics as a therapeutic strategy for respiratory diseases has still not been conclusively proven [87]. Consequently, it may be necessary to further broaden our horizons to fully understand the actions and mechanisms of probiotics in the prevention and treatment of respiratory tract diseases [87–89].

6. Bowel Mucus Composition

The mucus of the gastrointestinal tract, coating the intestinal epithelium, acts as a physical barrier, representing the first line of defense against ingested material, digestive enzymes and microbial by-products [90].

The mucus is synthesized and secreted by goblet cells and is mainly composed of water, lipids and mucins, a complex structure of glycoproteins with specific O-linked glycans (O-glycans) capable of guaranteeing the appropriate mechanical and viscoelastic properties [37,38,91,92]. Among the many mucins, Muc2 is the main mucin component of the intestinal mucus layer [93]. In addition to the mucins, mucus contains immune mediators that target the gut microbiota, providing a diffusion barrier. In fact, IgA, secreted into the lumen of the duodenum and the ileum at a high concentration, is able to bind bacteria or viruses, leading to slower diffusion and reducing bacterial mobility in the mucus [92].

The composition and thickness of the mucus vary along the length of the intestine. While in the small intestine, the mucus forms a single layer, in the large intestine, due to the higher density of microorganisms, the mucus is organized in two different layers: the inner one separates the commensal bacteria from the host epithelium and the outer one represents the real natural habitat for the commensal bacteria [94]. Moreover, in the small intestine, the mucus is penetrable, but the bacteria are kept away from the epithelium by antibacterial mediators, whereas in the large intestine, the inner mucus layer is impenetrable to bacteria and only the outer mucus layer hosts the bacteria [95]. Therefore, the inner mucus layer is denser and the outer is more dissolved, as it is exposed to the proteolytic activity of bacteria [91,96]. The mucus composition has an important role in creating a favorable microenvironment to host the gut microbiota, also providing nutrients and other physiological substances for its homeostasis [97]. For example, some bacteria, using MUC2 O-linked glycans, are capable of colonizing tissue and releasing a mucous biofilm, a complex self-produced polymer matrix in which microorganisms can attach to each other and be attached to the mucosal surface [98]. In addition, some bacteria can also use pili, fimbriae, or flagella to bind mucus [99,100]. Indeed, the main flagellar subunit protein has also been shown to be involved in bacteria–mucus adhesion. Finally, it is likely that the components of the mucus layer, such as secretory IgA and mucins, play a role in the interaction between bacteria and mucus [91,101].

7. Bowel Microbiota Composition

The gastrointestinal tract is one of the microbial ecosystems with the highest population density. It is estimated that about 10^{14} microorganisms are present in the intestine of an adult, exceeding the total number of cells in the human body (10^{13}), and including 100 times more genes than the human genome [102–104]. In general, the development of an individual's intestinal microflora depends on the number and type of microorganisms with which the individual comes into contact in the early stages of growth, and on the genetic composition of the individual [105]. The phenotype composition can therefore be regulated by such factors as diet, environment and stress. Infection, disease and antibiotics are among the many factors that may alter microbial composition [106].

The human intestinal microbiota contains bacteria, fungi, archaea, protists, helminths and viruses that symbiotically inhabit the human digestive system, with five bacterium phyla—Bacillota, Bacteroidota, Actinomycetota, Pseudomonadota and Verrucomicrobiota—representing the predominant microorganisms in the gut (Table 2) [2]. Along the intestinal tract, both the quantity and the quality of the microbiota vary. It is notable that bacterial concentration tends to increase along the distal ileum, reaching very high concentrations at the colon level. In addition, the horizontal stratification of the microbiota is also remarkable: different commensal microorganisms are located in the intestinal lumen, the mucus layer and the epithelial crypts [107].

Due to poor intestinal motility, the highest number of bacteria with the greatest microbial diversity are located in the large intestine (10^{11}–10^{12} CFU/mL of luminal content). This colonization allows the formation of a symbiotic and commensal relationship between the host and the microbiota, dependent on the production and the release of multiple bioactive metabolites [108,109]. The intestinal microbiota thus influences normal intestinal homeostasis, acting on the proliferation and apoptosis of intestinal epithelial cells (IECs).

Among the main metabolic functions performed by the microbiota, there are the synthesis of vitamins (B12 and K), absorption of Ca, Fe and Mg ions and the production of short-chain fatty acids (SCFAs) [110,111]. In general, SCFAs, such as butyrate, acetate and propionate, derived from the fermentation of indigestible fibers, play an important role in human physiology, maintaining intestinal barrier integrity by preventing microbial translocation, which is known to be associated with local intestinal inflammation, systemic inflammation and neuroinflammation [108,112].

The intestinal microbiota also plays an important role in the development and maintenance of the immune system [113], as confirmed by studies conducted on germ-free mice that were more sensitive to infections and had low IgA concentration [114–116]. In general, the immune system is a complex network of chemical and cellular mediators that can recognize any form of insult in order to keep the body healthy. However, it is important for the intestinal immune system to be able to recognize pathogenic microorganisms from commensal ones. In particular, during the development of the immune system, the Toll-like receptors (TLRs)—expressed on IECs and lymphoid cells—promote immunological tolerance to the normal components of the microbiota, recognizing several general molecules associated with microbes (i.e., microbe- or pathogen-associated molecular patterns—MAMPs or PAMPs, respectively), such as peptidoglycans and capsular components [117]. In this way, the intestinal microbiota not only manages to evade the host's immune system but also contributes to the development, maturation and regulation of the immune system [108].

Furthermore, although it is classically thought that the mucus produced by the goblet cells of the intestinal mucosa represents the first defense barrier, even the microbiota does not allow the colonization of exogenous/pathogenic microbes. In fact, there is competition between commensal and pathogenic bacteria. The colonization of exogenous bacteria can be prevented due to the limited availability of nutrients or to the production, during sugar metabolism, of organic acids that determine a reduction in intestinal pH with consequent inhibition of the growth of acid-sensitive bacterial species. Other than these mechanisms, one should also consider the production of antimicrobial substances by commensal bacteria, such as bacteriocins, allowing the microflora to control the growth of exogenous microorganisms [118].

It has been shown that the microbiota of the gastrointestinal tract is able to modulate the migration of the neutrophils resident in the lamina propria, to favor the differentiation of TCD8+ cells toward TCD4+, and finally to release IgA through the activation of B cells [119]. Hence, the complex interaction between nonpathogenic bacteria, IECs and immune cells of the mucous layer is a fundamental prerequisite for the development of immune functions and defense mechanisms in the adult intestine.

However, dysbiosis of the gut microbiota, associated with an increased abundance of potentially detrimental bacteria, can compromise gut barrier integrity through bacterial production of endotoxins (e.g., lipopolysaccharide (LPS)) capable of altering immune response, initiating proinflammatory pathways and directly damaging intestinal epithelial cells (known as leaky gut), with changes in the distribution and localization of its tight junction proteins. This promotes the translocation of bacterial components from the intestinal lumen to the systemic circulation and other organs, including the central nervous system (CNS). In fact, gut dysbiosis has also been observed in various neurological and psychiatric conditions, including severe depression and Parkinson's and Alzheimer's diseases [120]. For instance, several studies have reported the potential connection between α-syn-related pathology and dysbiosis [120,121].

It is thus widely understood that the microbiota communicates with the host under both physiological and pathological conditions. This type of communication takes place not only by direct contact but also through humoral signaling molecules and hormonal components. As previously described, intercellular communication with the host also occurs through the release of micro- and nanovesicles, which can enter the systemic circulation. In particular, the maintenance of tissue homeostasis is also mediated by the release of EVs, such as exosomes and OMVs/MVs, contributing significantly to coordinated signaling events and communication between microbiota, IECs, endothelial cells and immune cells [122]. Among proteins transported within the EVs, there are metalloproteinases, growth factors and chemokines, used as secondary messengers for the coordination of cellular responses [123]. Several studies have shown that the release of EVs could play a role in response to vaccination and therapeutic applications, as a vehicle in the delivery of drugs and targeted therapy [50,124–126]. In general, IEC-derived EVs are able to regulate the integrity of the epithelial barrier thanks to the transport of desmosomal cadherins that stabilize cell–cell epithelial adhesions, as well as being able to protect against pathogenic infections thanks to the transport of some antimicrobial peptides, such as beta-defensin [127,128].

In recent years, several studies have focused on the role of OMVs produced by the intestinal microbiota, highlighting their importance as immunological mediators. In fact, it has been shown that the large capsular polysaccharide A (PSA) is selectively packaged within OMVs. The OMVs are then internalized into dendritic cells, which induce the differentiation of T-regulatory cells to produce IL-10. This represents an example of how host immunotolerance towards the symbiote is determined [129,130].

Other studies show the entire intestinal microbial population benefits from OMV production A mutual support is established by the bacterial species that release OMVs and others that receive them. In fact, some bacteroides are able to pack hydrolases inside OMVs, making them usable to other bacteria that are privy to them. Since hydrolases are used for the digestion of polysaccharides, this mechanism promotes the growth of other bacterial species that are unable to hydrolyze polysaccharides. This again supports the role of OMVs in creating and maintaining the balance of the gut microbiota [131].

Table 1. The main bacteria that can be found in the airway microbiota. Percentages can change depending on age, lifestyle, epigenetic stimuli, and diseases [129].

Anatomy		Density	Kingdoms	Phylum	Genus	(%)	References
URT (upper respiratory tract)	Nasal cavity	10^3	Bacteria	Actinomycetota	*Corynebacterium* spp.	20–25%	[16,73,74,76–78,107,132–134]
				Bacillota	*Staphylococcus* spp.	7–12%	
					Streptococcus spp.	8.5–13%	
				Pseudomonadota	*Haemophilus* spp.	2–7%	
					Moraxella spp.	13–18%	
	Nasopharynx	10^3		Actinomycetota	*Corynebacterium* spp.	6–11%	
				Bacillota	*Staphylococcus* spp.	12–17%	
					Streptococcus spp.	10.5–15%	
				Pseudomonadota	*Moraxella* spp.	14–19%	
	Oropharynx	10^6		Actinomycetota	*Rothia* spp.	5.5–10%	
				Bacteroidota	*Prevotella* spp.	12–17%	
				Bacillota	*Streptococcus* spp.	23–28,5%	
					Lactobacillus spp.	1–5%	
					Veillonella spp.	4–9%	
				Fusobacteriota	*Leptotrichia* spp.	4–9%	
LRT (lower respiratory tract	Lungs	10^2	Bacteria	Bacteroidota	Prevotella spp.	75–80%	
				Bacillota	*Lactobacillus* spp.	78–83%	
					Streptococcus spp.		
					Veillonella spp.		
				Pseudomonadota	*Halomonas* spp.	41–46%	

Table 2. The main bacteria that can be found in the bowel microbiota. Percentages can change depending on age, lifestyle, epigenetic stimuli, and diseases [135].

Domain	Kingdoms	Phylum	Genus	(%)	References
Procaryota	Bacteria	Bacteroidota	Bacteroides Prevotella	73.13 ± 22.16%	[136–139]
		Bacillota	Clostridium Lactobacillus	22.2 ± 18.66%	
		Actinomycetota	Bifidobacterium	1.67 ± 2.94	
		Fusobacteriota	Fusobacterium	19 ± 21.4%	
		Pseudomonadota	Escherichia Shigella	2.15 ± 10.39%	
		Verrucomicrobiota	Akkermansia	1 ± 2%	
		Spirochaetota	Brachyspira	5 ± 7.8%	
	Archea	Euryarchaeota	Halobacterium	0.1 ± 21.3%	[140,141]
Domain	**Kingdoms**	**Phylum**	**Family**	**(%)**	**References**
Eukaryota	Fungi	Ascomycota	Aspergillaceae Debaryomycetaceae Dipodascaceae Saccharomycetaceae	47.8 ± 99.5%	[142–144]
		Basidiomycota	Tremellaceae Malasseziaceae	47.8 ± 99.5%	
Virii	Bacteriophage	Uroviricota	Demerecviridae Ackermannviridae Autographiviridae	80 ± 84%	[139,145–147]

8. Conclusions

The MuMi layer of the airways and bowel should not be misinterpreted any longer: its identification and study can enable a better understanding of the physiology and pathophysiology of these organs and, in turn, of the entire organism. Further studies are essential to better gauge not only the microbe population of the MuMi layer (both in qualitative and quantitative terms) but also the mucous component, i.e., the "matrix" in which these microbes live, proliferate, and traffic soluble molecules and nanovesicles during the entire life span of an individual, both in physiological and pathophysiological conditions. A better understanding of this important constituent of the human body could lead to the enhancement of diagnostics and treatment strategies for airway and bowel diseases, as well as their extraorgan complications.

Author Contributions: Conceptualization, F.B. and F.C.; software, S.B.; writing—original draft preparation, A.F., S.B., L.P., C.C.B., A.P., F.B. and F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: Not applicable

Acknowledgments: We thank Gabriele Chiaramonte (University of Palermo) for microbiological advice.

Conflicts of Interest: The authors declare no conflict of interest.

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