Trends in Cancer

CellPress REVIEWS

Opinion

The Influence of Lung Microbiota on Lung Carcinogenesis, Immunity, and Immunotherapy

Ariel G. Ramírez-Labrada,[1] Dolores Isla,[2] Angel Artal,[3] Maykel Arias,[8] Antonio Rezusta,[4,5] Julián Pardo,[5,6,7,9,*] and Eva M. Gálvez[8,9,*]

Microbiota have emerged as key modulators of both the carcinogenic process and the immune response against cancer cells, and, thus, it seems to influence the efficacy of immunotherapy. While most studies have focused on analyzing the influence of gut microbiota, its composition substantially differs from that in the lung. Here, we describe how microbial life in the lungs is associated with host immune status in the lungs and, thus, how the identification of the microbial populations in the lower respiratory tract rather than in the gut might be key to understanding the lung carcinogenic process and to predict the efficacy of different treatments. Understanding the influence of lung microbiota on host immunity may identify new therapeutic targets and help to design new immunotherapy approaches to treat lung cancer.

Highlights

Lungs are no longer considered sterile and their microbiota are associated with lung wellness.

The lung microbiome has been linked to lung carcinogenesis and establishment of lung metastasis from other primary cancers.

Lung microbiota dysbiosis may modulate the risk of malignancy at multiple levels including chronic inflammation and oncogenes.

Patients treated with antibiotics before/during immunotherapy present with significantly lower progression-free survival and overall survival rates compared with patients who have not received antibiotics.

Profiling of the gut microbiota revealed dysbiotic signatures associated with delayed tumor outgrowth and favorable responses to immunotherapy.

An Overview of the Connection between Host Microbiota and Cancer

The relationship between microbiota (Box 1) and cancer is currently under intensive investigation. Although microbiota are composed of bacteria, archaea, protists, fungi, and viruses, most studies addressing the influence of microbiota on cancer have focused on bacterial microbiota, and thus we also focus on bacteria. A large number of studies providing insights on how bacteria and abnormal growth of mammalian cells are related have been conducted. Bacteria may disrupt the cell cycle by toxin production, resulting in cell growth with alterations in protein expression that control DNA repair, cell division, and apoptosis [1,2]. Furthermore, bacteria may alter the host immune response against malignant cells, and an association between microbiota composition and clinical immunotherapy response has recently been shown. Studies in animal models indicate that microbiota modulate the sensitivity of solid cancers to immune checkpoint inhibitors (ICIs), mainly cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed cell death-1 (PD-1)/ligand 1(L1) [3–5]. The best characterized microbiome–cancer relationship is between gut microbiota and gastrointestinal and metabolic diseases such as gastric cancer [6], inflammatory bowel disease [7] (a risk factor for colorectal cancer), diabetes, and obesity [7–9]. The relationship between gut-related pathogenesis and microbiota is not surprising since the gut is the main tissue colonized by the commensal microorganisms, which comprise 3.93×10^{13} bacteria. However, during recent years it has been found that other healthy organs and tissues also contain significant numbers of microorganisms, such as the lower respiratory tract. Thus, the principles of respiratory microbiology are being studied again, starting with the lung sterility myth [10]. For example, the association between oral microbiota and risk of lung cancer has been reported [11]. Lung cancer is currently one of the most common causes of cancer death worldwide in both men and women. The 5-year survival rate of lung cancer patients is <20% [12]. Thus, understanding how the microorganisms present in the respiratory tract might influence lung carcinoma development and treatment efficacy could be key in predicting the risk of cancer development

[1]Unidad de Nanotoxicología e Inmunotoxicología (UNATI), Instituto de Investigación Sanitaria Aragón (IIS Aragón), Centro de Investigación Biomédica de Aragón (CIBA), Zaragoza, Spain
[2]Medical Oncology Department, Instituto de Investigación Sanitaria Aragón, Hospital Clinico Universitario Lozano Blesa, Zaragoza, Spain
[3]Medical Oncology Department, Instituto de Investigación Sanitaria Aragón (IIS Aragón), Hospital Universitario Miguel Servet, Zaragoza, Spain
[4]Department of Microbiology, Hospital Universitario Miguel Servet, Zaragoza, Spain

[5]Department of Microbiology, Preventive Medicine and Public Health, University of Zaragoza, Zaragoza, Spain
[6]Aragón Health Research Institute (IIS Aragón), Biomedical Research Centre of Aragón (CIBA), Zaragoza, Spain
[7]Aragón I + D Foundation (ARAID), Government of Aragon, Zaragoza, Spain
[8]Instituto de Carboquímica ICB-CSIC, Zaragoza, Spain
[9]These authors share senior authorship.

*Correspondence:
eva@icb.csic.es (E.M. Gálvez).

Box 1. Microbiota and Microbiome

The term microbiota refers to the ensemble of microorganisms (bacteria, archaea, fungi, viruses, and protozoa) that reside in an individual at a given time, whereas the total genome of these microorganisms is designated as the microbiome [70,71]. Microorganisms are found in many parts of the human body, primarily on the external and internal surfaces, including the gastrointestinal tracts, saliva, oral and genital mucosa, lung, bladder, skin, and conjunctiva. The human microbiota is primarily colonized by Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, and Cyanobacteria [1,72–74]. Currently, it has been estimated that a ratio of bacteria to human cells in the body is close to 1:1 [75].

and to improve treatment efficacy and safety. The human body is teeming with microbes, but the composition of the microbiota differs by anatomical site, such as the oral and nasal cavities (interconnected to the lung and the stomach via the pharynx and esophagus, respectively) colon, vagina, and skin. Cooperative interactions between microbiota and host might involve microbial participation in host functions such as defense and metabolism. Thus, the same microorganisms that are beneficial to human health, under certain circumstances, might promote disease and cancer development. In other cases, changes in the composition of microbiota might lead to disease. (See Table 1.)

In this review, we summarize the current scientific knowledge to gain a deeper insight into how the lung microbiota composition and function may affect lung cancer development. We discuss how lung microbiota changes could alter host–microbiota interactions by modulating the healthy and pathological immune response and how this modulation offers new alternatives for successful and safe immunotherapy in lung cancer.

Lung Microbiota

Viable and Nonviable Components of Lung Microbiota

The lungs are constantly exposed to microorganisms present in the upper respiratory airways and suspended in the air. Nevertheless, until recently, the healthy lungs were considered to be sterile organs since bacteria were rarely isolated from normal healthy lungs using conventional culture techniques. However, although bacterial biomass in human lungs is low (5–8.25 log copies/ml) [13,14], nowadays bacterial DNA is commonly found in the lower respiratory tract, where it has been detected in healthy individuals thanks to next-generation sequencing

Table 1. Bacterial Communities Detected in Lung Cancer Patients

Taxa features	Sample numbers	Sample type	Refs
Granulicatella *Abiotrophia* *Streptococcus*	16	Sputum samples	[35]
Granulicatella *Streptococcus* *Mycobacterium*	10	Sputum samples	[38]
Veillonella *Megasphaera*	28	Bronchoalveolar lavage	[37]
Cyanobacteria	29	Lung tissue	[1]
Streptococcus *Neisseria*	42	Protected bronchial brushing	[15]
Streptococcus *Prevotella*	40	Lung tissue	[36]
Acidovorax	176	Lung tissue	[17]
Streptococcus *Veillonella*	85	Airway brushes	[32]

Box 2. Molecular Methods to Characterize the Lung Microbiota

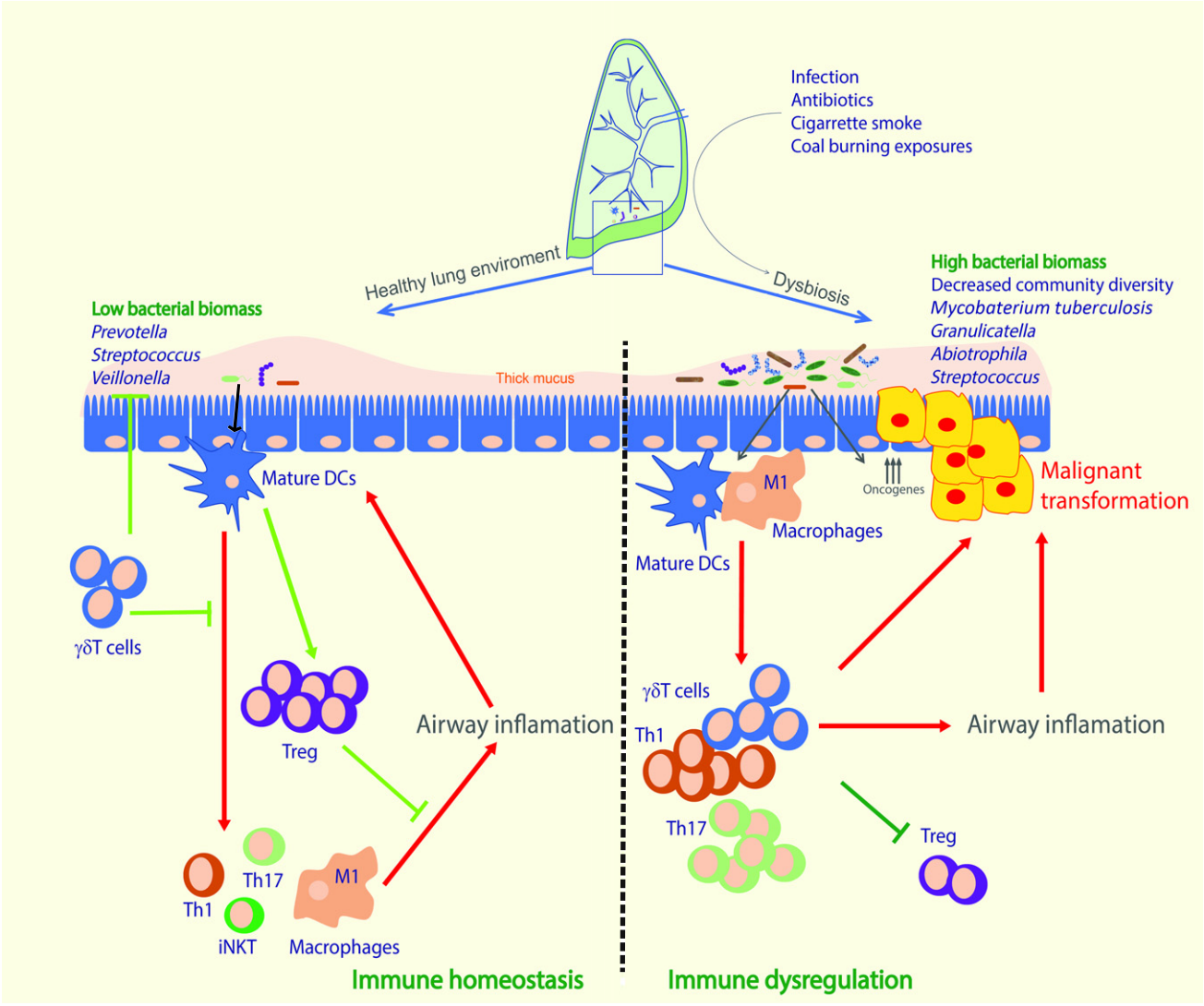
NGS has emerged as a potent technology for the molecular characterization of microorganisms in complex samples. According to the desired microbial kingdom to be characterized, bacteria, archaea, or fungi, specific primers against genomic regions conserved within every kingdom are selected. Primers against the 16S rRNA, 18S rRNA, or internal transcribed spacer (ITS)1/ITS2 regions and V4/V9 regions in 18S rRNA are selected for bacteria/archaea, fungi, and protists, respectively. In the case of viruses, purification of virus-like particles followed by shotgun technology is preferred [76].

By far, 16S rRNA sequencing is the most advanced technology and has been key to identify the prokaryotic (bacteria and archaea) microbial composition of complex samples. However, it still presents some limitations, such as its inability to differentiate between species with varying immunogenicity and pathogenicity. 16S rRNA sequencing only provides information at the genus level and does not differentiate species since this technology is based on short read lengths, which makes precise taxonomic assignment difficult. This challenge potentially explains why the most robust associations that have been observed between bacterial species and the human gut concern species that are unique in humans among their genus [77–79]. An alternative to directed NGS, is whole genome sequencing, which may be even more informative [80]. All these recent developments have enabled identification beyond the species level, but only for dominating populations. Major advances in bioinformatics and combination of culture-dependent and -independent methods like matrix-assisted laser desorption/ionization (MALDI)-TOF (time of flight) mass spectrometry and 16S rRNA sequencing has enabled the analysis of entire sequence data sets using similarity algorithms or clustering tools without taxonomic assignment [79,81,82]. This has allowed the discovery of unknown bacteria [79] and the isolation of hundreds of new bacterial species in less than 5 years. Despite all these advances in molecular identification and diagnosis, it is worth noting that culture-independent methods are not always more sensitive than traditional culture methods for the identification of bacterial species. For example, 16S rRNA sequencing has shown lower performance than culture in the identification of species within the *Mycobacterium* genus [83]. Thus, it seems that a combination of molecular culture-independent methods with culture might be the best option for reliable characterization of bacterial populations and microbiota.

(NGS) technologies (see Box 2) [13,15–18]. It should be noted that different studies suggest that most bacterial DNA detected in the lungs might come from nonviable bacteria. Indeed it has been found that more than 90% of microbial DNA was DNase I sensitive, indicating that this DNA originated from nonviable microorganisms [16,19]. Willis *et al*. also found that up to 50% of the bacterial DNA from sinus tissue was derived from nonviable sources [20]. The traditional view that viable bacteria are rarely present in normal healthy lungs is consistent with the lungs being actively surveilled by immune cells and the observation that 30% of tissue samples were sterile in the Scheiermann and Klinman study [16]. In line with these findings, Segal *et al*. reported that the bacterial DNA content in some human lung samples could not be distinguished from that of background/negative control samples [18]. These findings supported the conclusion that most of the bacterial DNA isolated from lung tissue derives from dead/nonviable organisms. Bacterial DNA could be biologically relevant in shaping the lung immune system since CpG motifs present in bacterial DNA can activate the innate immune system via Toll-like receptors [21,22] or other pathogen recognition receptors found in alveolar macrophages and bronchial and alveolar epithelial cells [22,23]. In contrast to the findings mentioned above, independent studies have reported that the microbes present in the lower respiratory tract can be cultured when appropriate protocols are utilized, indicating that this microbiome is not simply composed of bacterial DNA or debris [24,25].

Initial Establishment of Lung Microbiota

Studies in animal models have demonstrated that bacterial load in the lungs increases over the first 2 weeks of life, and the taxa of organisms detected in the lung change from Gammaproteobacteria and Firmicutes to Bacteriodetes [26]. Such microbiota changes are thought to be associated with accumulation of a PD-L1-dependent T regulatory cell population that promotes tolerance to environmental allergens [26]. Therefore, the lung microbiota would be a key early life event required to shape the lung immune system and protect the organ from injurious inflammatory responses to inhaled antigens. This early process might promote commensal microbiota tolerance, avoiding inflammation-related lung damage and, likely, lung



Trends in Cancer

Figure 1. Relationship between Lung Microbiota, Homeostasis, and Lung Cancer. There is a delicate equilibrium between the immune system and microbiota. The commensal microbiota contributes to immune tolerance, decreasing lung inflammation through dendritic cell (DC), γδ T, and T regulatory (Treg) cell recruitment. Macrophages and T cells respond to microbial colonization and prevent the overload of pathogens or metabolites (left panel). Lung microbiota might contribute to lung cancer development. Bacteria might promote proinflammatory factors leading to chronic inflammation and upregulation of proliferative signaling pathways in airway epithelial cells inducing cell transformation and tumorigenesis (right panel). In addition, some microbial components might directly affect protumorigenic pathways in epithelial cells (oncogenes). Abbreviations: iNKT cell, invariant natural killer cell; Th1/17, T helper type 1/17.

carcinogenesis (Figure 1). In humans, it has been recently shown that the microbial communities in the lower airways are acquired within the first 2 months of life [27]. Lung microbiota composition depends on the delivery method in preterm but not term infants. Thus, these data suggest that diseases related to lung microbiota composition such as cancer might be influenced by the duration of pregnancy, although this hypothesis requires validation. Nevertheless, similar to the upper respiratory tract and gut, it is likely that the bacterial communities in the lung are dynamic [28,29], since the airways are constantly exposed to air that flows through the upper respiratory tract and oral cavity. Thus, in healthy individuals, it is not clear to what extent the deep airways are stably colonized by specific microbial communities, or whether the microbes are in a dynamic

state of flux being constantly cleared and repopulated, most likely from the upper respiratory tract. At least, under healthy conditions, it seems that there is constant seeding and turnover of the lower respiratory tract microbiota, which can be characterized by both culture-dependent and -independent methods [30].

Microbiota Composition of Healthy Lungs

The microbial composition in healthy lungs analyzed by culture-independent techniques seems to be enriched in the phyla Bacteroidetes and Firmicutes [18,31]. It was found that oral commensals like *Prevotella*, *Veillonella*, and *Streptococcus* are present in the lungs from most healthy individuals [13,15,18,31,32]. Microaspiration of pharyngeal secretions in healthy subjects seems to be the main source of the lung microbiome [2–5]. However, these genera might not be present in all healthy individuals as it has been shown that the lung microbiome can be classified according to the bacterial load and taxa in two groups called pneumotypes. The first group has a high bacterial load and is enriched with oral microorganisms such as *Prevotella* and *Veillonella*, and is known as the supraglotic predominant taxa (SPT) pneumotype. The second group has a low bacterial load and background environmental taxa such as *Acidocella* and *Pseudomonas*, and is known as the background predominant taxa (BPT) pneumotype [18]. While individuals exhibit some spatial variation in the microbiota of their respiratory tract, intrasubject variation is significantly less than that of intersubject variation [13].

It has been shown that the bacteriome enriched in oral commensals (SPT) correlates with T helper 17 cells (Th17)-related local inflammatory response, which seems to be key in modulation of lung immune status in health and disease (Figure 1) [33]. It will be interesting to find out whether individuals with the STP pneumotype present with a higher or lower risk of lung cancer or if changes from the BTP to STP pneumotype affect the risk of lung cancer development and/or the efficacy of treatment. This is not a trivial question since the correlation between Th17 response and cancer is not clear yet, as discussed later.

Another study of nonmalignant lung tissue indicated that the lung microbiota differ from those of the oral cavity and other sites and are dominated by *Proteobacteria* (60%) [34].

Microbiota Composition in Lung Carcinoma

Concerning the lung microbiota in lung cancer patients, although few studies have been performed, a significant enrichment of *Granulicatella*, *Abiotrophia,* and *Streptococcus* at genus level and decreased community diversity have been observed in patient compared with control samples [15,34–38]. The difference in the bacterial communities between healthy individuals and lung cancer patients might serve as a screening tool to predict lung cancer development using bronchoalveolar fluid. Liu *et al.* have provided evidence that lung-cancer-associated microbiota are enriched in *Streptococcus* while depleted in *Staphylococcus*, which suggests a deleterious role of *Streptococcus* and protective role of *Staphylococcus* in the development of lung cancer [15]. This hypothesis contrasts with other findings about the role of these taxa in carcinogenesis, since it has been shown that *Staphylococcus* has the ability to induce DNA damage while *Streptococcus* may play a role in its prevention [39]. However, these apparent contradictory findings could be explained by the difficulties in identifying actual species or strains involved in carcinogenesis. By contrast, certain taxa might play distinct roles at different body niches or even the same taxa can have protective or detrimental functions at the same place depending on the presence of different stimuli.

It should be considered that the lung microbiota composition are associated with lifestyle, pollution, tobacco smoke, and coal burning, and these factors might have contributed to the different

results reported. There are also differences in patients with chronic bronchitis or tumors [34,35]. It has been shown that the genus *Thermus* is more abundant in tissue from advanced stage cancer patients, while *Legionella* is more abundant in people who develop metastases [34]. However, more and larger studies, both in animal models and patient lung tissues, are necessary to validate these findings, and to gain deeper knowledge of the composition of lung microbiota and their role in lung cancer, before they can be used as cancer biomarkers or included in therapeutic approaches.

Microbiota Composition Modulates Chronic Inflammation and Cancer Development

The lung microbiome has received less attention than the gut microbiome, and, by contrast to gastrointestinal cancer, the correlation between microbiota and lung cancer has been less studied. However, even though there are fewer studies, the composition of the lung microbiota has recently been associated with lung cancer. Adenocarcinoma and squamous cell carcinoma are the major forms of non-small cell lung cancer (NSCLC). Even when smoking is correlated with its etiology, it alone cannot completely explain lung cancer incidence. Some authors have hypothesized that altered lung microbiome and chronic inflammation in lung tissue contribute to carcinogenesis [1,17]. In this regard, the correlation between repeated antibiotic exposure and increased risk of lung cancer has been studied. Although there is epidemiological evidence pointing out this relationship, the contribution of the lung microbiome to lung cancer is still unknown [40]. It is well known that chronic inflammation is a risk for cancer development, including lung cancer [41], and this relationship has been the focus of most studies linking microbiota and lung carcinogenesis, as discussed in detail in the next section. Several bacteria have been associated with chronic inflammation and subsequent increased cancer risk. For example, *Mycobacterium tuberculosis* has been associated with lung cancer [42], *Bacteroides fragilis* and *Fusobacterium nucleatum* to colon cancer, and *Helicobacter pylori* to gastric cancer.

Bacterial Products Might Promote Host Oncogene Activation

Some microbial components might directly activate molecular pathways with oncogenic potential. Although few microbes have been identified as carcinogenic *per se*, with the notable exception of some oncoviruses, recent work has identified some relationships between changes in the lung tissue microenvironment and microbial colonization that might affect cell transformation and carcinogenesis. Greathouse *et al.* hypothesized that the interplay between smoking, TP53, and microbiota might be relevant during smoking-driven lung carcinogenesis. Lung epithelial cells with mutations in TP53 due to tobacco smoke are invaded by species that take advantage of the new microenvironment, suggesting that these bacteria could act as promoters in lung tumorigenesis [17]. Another study showed that ERK and phosphoinositide 3-kinase (PI3K) signaling pathways are upregulated *in vivo* and *in vitro* after exposure of airway epithelial cells to *Veillonella*, *Prevotella*, and *Streptococcus* [32]. PI3K is a key pathway involved in the pathogenesis of NSCLC since it regulates cell proliferation and survival [2,43]. Apopa *et al.* found that CD36 could act as the connection between lung microbiota and specific insults that contribute to lung cancer development [1]. Altered expression of CD36 in lung tissue is associated with lung cancer [44–46]. CD36 has been shown to interact with pathogen-derived ligands or toxins [47,48] and it is an important mediator of inflammatory pathways [49]. However, they showed that CD36 might modulate lung carcinogenesis by affecting the poly (ADP-ribose) polymerase 1 (PARP1) pathway [49] that is an important regulator of cell proliferation and carcinogenesis. It was found that CD36 regulates the internalization and processing of *Cyanobacteria*-derived microcystin residues in the lung alveoli, increasing PARP1 expression [1]. They detected, in addition to Bacteriodetes and Proteobacteria as the most predominant phyla, Cyanobacteria (0.53%) in patient lung samples, supporting the relevance of the mechanism described.

Lung Microbiota, Immunosurveillance, and Immunotherapy

Microbiota Shapes a Healthy Lung Immune System

Lung microbiota are thought to provide resistance to colonization by respiratory pathogens and to play a key role in the regulation of immune tolerance in the lung microenvironment (Box 3). In addition, they are considered integral to the development and training of the human immune system. It is likely that the relationship between our pulmonary immune system and the microbiota is dynamic and this relationship likely changes with age and environmental exposures, in line with the dynamic changes observed for the lung microbial communities as mentioned earlier. The coevolution of the host immunity–microbiota interaction is likely to be responsible for the development of regulatory pathways that modulate self-tolerance and tolerance against nondangerous agents versus elimination of pathogens and tumor cells [50].

In the lungs, γδ T cells are thought to be important effector and regulator cells in host innate immune responses to pulmonary infections, and their precise role seems to be pathogen dependent [51]. Inhalation of innocuous bacteria, which do not result in a bacterial dysbiosis or infection, has been shown to promote recruitment of γδ T cells and protect against airways hyper-responsiveness. In addition, γδ T cells appear to have a protective effect against allergy (Figure 1) [52–54]. Remot *et al.* have also shown that colonization of the neonatal airways with specific bacterial strains protects against exaggerated allergic airway inflammation [24]. Data from experimental models have confirmed that microbial components can protect mice against allergic airway inflammation developing tolerance to aeroallergens [26,55]. These and other works suggest that microbial exposure throughout childhood is required for the generation of a healthy fully competent lung immune system [26,52,54–57]. Invariant natural killer (iNK) T cells and PD-L1-tolerogenic pathways on dendritic cells (DCs), which regulates the induction of peripheral regulatory T (Treg) cells, seem to be the critical cellular mechanisms that modulate these processes (Figure 1).

Box 3. Lung Immunity

The upper and lower respiratory tracts are critical sites where the host immune system must respond against potentially harmful agents, differentiating them from self-components, foreign nondangerous material, and beneficial commensal microbiota. Among the intricate organization of local immune cells responsible for maintaining lung homeostasis, the epithelium–macrophage axis is a key part that facilitates maintenance of the steady state. The pulmonary epithelium is a barrier that provides a vital line of host defense against pathogenic microorganisms. Within the epithelium, ciliated columnar, mucus secreting goblet cells, tuft cells, and Club cells form a regulated, impermeable barrier. The airway epithelial layer has another protective feature which is mucociliary action [84,85].

Although epithelial cells and macrophages make-up the first line of defense of the airways, there is a second line, consisting of tissue-resident lymphoid cells. This resident population is mainly formed by γδ T cells, NKT cells, innate lymphoid cells (ILCs), and specific subsets of memory B and T cells, the resident memory B and T cells. When an antigen is encountered in the lungs, together with resident memory cells, specialized subsets of T and/or B cells are activated in draining lymph nodes and recruited to the lung to participate in the elimination of the offending insult.

Activation of the adaptive immune response is regulated by an integrated network of DCs that can roughly be divided into two subsets, the $CD11b^{+}CD103^{-}$ and $CD11b^{-}CD103^{+}$ DCs [86–88]. Macrophages and DCs are some of the most critical determinants of the immunological tone of the airways.

Lung-resident memory T cells, characterized by expression of CD4, CD8, CD69, and a diverse T cell receptor repertoire, form an important component of adaptive immunity at barrier surfaces and provide rapid immune responses in healthy lungs [89–91]. The presence of different ILC subsets is enhanced at barrier surfaces and they are thought to play a key role for maintenance of homeostasis, and regulation of immunity and tissue repair [92]. In the same way, Treg cells are resident cells within lungs and are vital for maintenance of immune tolerance to airborne particles by a PD-L1-dependent mechanism [93]. Accumulating evidence shows that these resident pulmonary Treg cells are present from birth and their phenotype is directly influenced by the local microbiota [26]. γδ T cells constitute a major T cell component of mucosal epithelial barrier tissues where they can respond to danger signals and facilitate orchestration of immune responses [94,95].

Microbiota and Cancer Immunosurveillance

The delicate balance between tolerance and lung immune activation can be disrupted by changes in the host immunity–microbiota partnership by the overuse of antibiotics, changes in diet, or chronic infections, which might increase the risk of lung cancer [58]. The potential increased risk of lung cancer due to changes in microbiota could be related to either hyper-responsive immunity leading to chronic inflammation as described in the previous section (Figure 1) or defective immunosurveillance mechanisms (Figure 2).

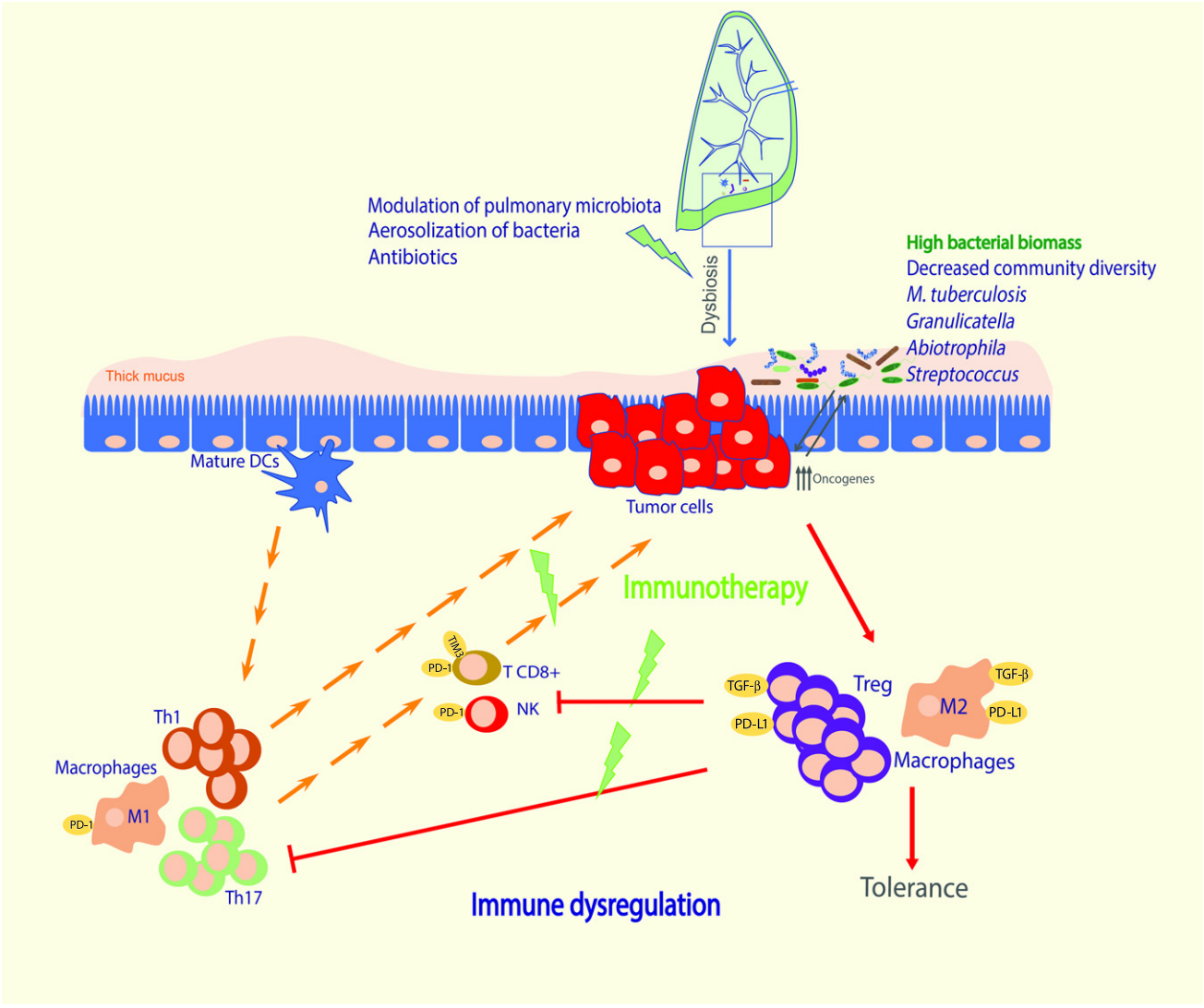


Figure 2. Potential Immunomodulatory Approaches to Revert the Protumoral Immune Microenvironment Due to Lung Microbiota Dysbiosis. Red lines: tumor development shapes an immunosuppressive microenvironment enriched in T regulatory (Treg) cells and M2 macrophages expressing anti-inflammatory molecules such as programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), or transforming growth factor TGF-β. These factors inhibit antitumoral natural killer (NK) and T cell responses, promoting tolerance and tumor immune evasion. Lung microbiota dysbiosis and the presence of specific bacterial strains might contribute to the generation of this immunosuppressive microenvironment and the low efficacy of immunotherapy treatments. Green arrows: immunotherapy treatments such as immune checkpoint inhibitors (ICIs) in combination with strategies to modulate lung microbiota (bacterial aerosols and/or selective antibiotic treatments) might help to revert dysbiosis and enhance antitumoral host immune responses. Abbreviations: DCs, dendritic cells; PD-1, programmed cell death-1.

Regarding defective immune response, Cheng *et al.* demonstrated the importance of commensal bacteria in supporting the host immune response against cancer, revealing a defective induction of lung immunity after antibiotic treatment [59]. Notably, in the same way that healthy microbiota modulate tolerance and are beneficial to prevent allergic reactions, under some circumstances, they might create a permissive environment for cancer. For example, some bacteria might help cancer cells to colonize lung tissue and establish lung metastases. Le Noci *et al.* found that local antibiotic treatment reduces the implantation of experimental lung metastases and this effect is associated with the modulation of the immune response [60]. Thus, these aspects should be carefully considered before any treatment using different bacteria strains are implemented in clinical settings.

An exacerbated immune response might lead to chronic inflammation and cancer development. The immune response induced by microbiota would be beneficial or deleterious to cancer patients, likely depending on the specific types of responses activated and the specific types of bacteria involved (Figures 1 and 2). For example, bacteria-driven immune activation may exacerbate tumorigenic inflammation (Figure 1). Ma *et al.* found that NSCLC patients presented significantly higher frequencies of T helper type 1 (Th1) and Th17 cells reacting to *Streptococcus salivarius* and *Streptococcus agalactiae* compared with healthy controls [61]. Importantly, lung inflammation mediated by Th17 cells has been identified as an important factor in the initiation and metastasis of lung cancer [61,62]. However, this finding should be interpreted with caution since it has been shown that Th17-mediated neutrophil responses either promote carcinogenesis or, in contrast, can protect from cancer development and contribute to treatment efficacy [63,64].

Notably, γδ T cells have been recently found to contribute to lung cancer development after enhanced activation and proliferation mediated by commensal lung microorganisms [65]. The amount of lung bacterial load is directly linked to lung cancer development due to enhanced inflammation. This process is mediated by myeloid cells that enhance γδ T cell activation and proliferation (Figure 1) [65,66]. In contrast to carcinogenic inflammatory immunity, specific $CD8^+$ T cell-dependent inflammation toward certain bacterial strains has been demonstrated to assist traditional chemotherapy by enhancing the immune responses in mouse experimental models (Figure 2) [67,68]. This finding is supported by the demonstration that the aerosolization of bacteria isolated from lung microbiota of antibiotic-treated mice reduces lung metastasis implantation by enhancing cancer immune response [60]. This study also showed that lung microbiota might be manipulated by antibiotic or probiotic aerosolization, and those changes are associated with a reversion of immunosuppression present in the tumor microenvironment (Figure 2), favoring the immune response against cancer cells. In addition, this treatment increased the activation of the antitumoral NK and T cell response and the maturation of resident antigen-presenting cells (APCs), correlating with a reduction of protumoral M2 macrophages and Treg cells [60]. It was also found that after the shift from Firmicutes to Proteobacteria as the main Gram-negative bacterial species, two ubiquitous opportunistic pathogens from the Proteobacteria phylum, M*organella morganii* and *Escherichia fergusonii*, exerted an immunostimulatory effect through the production of different virulence factors [60]. These results confirm the key role of lung bacteria in the antitumor immune response and advocate that a balance among different bacterial species is crucial for antitumor immune responses. Importantly, they support the use of aerosolization with probiotics or antibiotics as a clinical therapeutic procedure to improve patient outcome [60].

Microbiota and Cancer Immunotherapy

Most experimental studies analyzing the role of microbiota in the efficacy of immunotherapy have been focused on gut microbiota, indicating that specific bacterial taxa are associated with the

antitumoral effect of ICIs, anti-CTLA4, and anti-PD-1/L1 [3–5,67] or with the efficacy of cyclophosphamide in lung cancer patients [68]. One of those studies showed that patients treated with antibiotics early before, during, or shortly after treatment with ICIs significantly decreased their efficiency against advanced epithelial cancers, including lung cancer [3]. Although this study was focused on changes in gut microbiota during antibiotic treatment, it is expected that those changes also affect lung microbiota and, thus, might modulate the local immune response against lung carcinoma. However, this assumption should be experimentally validated. Derosa *et al.* also showed that previous antibiotic treatment was associated with worse outcomes in NSCLC patients treated with ICIs, although changes in lung microbiota were not analyzed [69].

Concluding Remarks

Lung cancer is the leading cause of cancer deaths worldwide and while smoking and chronic obstructive pulmonary disease (COPD)/emphysema is a well-established risk factor for lung cancer, only a small percentage of these patients develop lung cancer. Evidence now supports that the lung microbiota may play a key role in carcinogenesis and in the response to chemotherapy and immunotherapy (Figure 2). Even though airway microbiota are clearly low in terms of biomass when compared with gut microbiota, we should not underestimate the potential significance of these local host–microbe interactions. Several studies have recently reported a correlation between specific components of lung microbiota, lung carcinogenesis and metastasis, and the lung immune response. These interactions include the modulation of chronic tumorigenic inflammatory responses, the appearance of genetic alterations driven by specific bacterial components and the generation of defective local immune responses.

Recent studies have shown that the composition of gut microbiota influences the cancer immune response and the efficacy of ICIs in different types of cancer. However, it is still dependent on experimental validation if, as expected, the composition of lung microbiota also regulates the efficacy of ICI immunotherapy in lung cancer patients (Figure 2, see Outstanding Questions). This hypothesis is supported by evidence from studies including lung cancer patients treated with antibiotics and ICIs, and the correlation between lung microbiota composition and lung immune responses. However, it should be pointed out that the effect of antibiotics observed in gut microbiota might be different to those observed in lung microbiota, in light of the intrinsic physiological differences of both tissues. In addition, we do not yet know if, as in the case of gut inflammatory toxicity observed during ICI immunotherapy, lung inflammatory pneumonitis observed during ICI treatment also depends on the presence of specific microorganisms in the lungs (see Outstanding Questions).

Proper analyses correlating lung bacterial microbiota composition, antibiotic treatment and/or ICI efficacy, and toxicity will be required to reach this conclusion (see Outstanding Questions) and support the use of therapeutic approaches such as antibiotics or aerosolized microbial compositions, to modulate lung microbiota and improve the treatment of lung cancer.

Once we confirm all these hypotheses, new challenges will require further optimization, such as monitoring the length and degree of dysbiosis, that will depend on antibiotic nature and presence of resistant bacteria, which will dictate the timing and composition of microbiota recolonization (see Outstanding Questions).

Last but not least, due to the general use of NGS directed against 16S, most studies have focused on the bacterial component of microbiota. However, it is expected that other microbial communities such as fungi and viruses might also influence the lung carcinogenic process as

Outstanding Questions

What is the best method to analyze the lung microbiome?

How and when does the lung microbiota affect lung cancer development?

Are there oncobacterial strains capable of producing substances with oncogenic properties?

How does the host immunity–bacteria interaction boost cancer development?

Could the lung microbiota profile be used as a predictive biomarker for risk of lung cancer development and/or for patient outcome (response and/or toxicity) during immunotherapy?

What bacterial taxa may have a beneficial and/or detrimental effect on lung carcinogenesis?

Could specific lung commensal bacteria be modulated to prevent lung cancer development?

Could the lung microbiota be modulated to improve response and reduce toxicity in lung cancer patients treated with immunomodulatory agents?

well as the natural and pharmacological modulation of the immune response in lung carcinoma (see Outstanding Questions). All these studies will require time and effort but the final goal deserves it.

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