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Features of the lung microbiota in tuberculosis infection

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ABSTRACT

Normal lung microbiota is a small number of transient microbes; however, respiratory pathology may be associated with persistent microbial colonization of the lungs. It remains a poorly understood and mysterious part of the pathogenesis of tuberculosis infection.

The review considers the general pathogenetic mechanisms of the effect of lung microbiota in respiratory pathology and presents the main methodological difficulties in the study of the lung microbiome. This review is aimed at analyzing the results of the available studies on diverse microbial composition of human lungs in tuberculosis using metagenomic sequencing methods. Despite high variability of the presented data, we can conclude that dysbiosis in tuberculosis is more often characterized by a decrease in bacterial diversity and enrichment of lung microbiota with anaerobic bacteria. *Acinetobacter*, *Campylobacter*, *Moraxella*, *Pseudomonas*, *Staphylococcus,* and *Streptococcus*, as well as some other microorganisms are indicated as important pathogenetic factors of dysbiosis in pulmonary tuberculosis, the role of which is yet to be elucidated.

Keywords: microbiota, microbiome, lungs, tuberculosis, metagenomics

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Особенности микробиоты легких при туберкулезной инфекции

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РЕЗЮМЕ

Микробиота легких представляет собой маятник, который колеблется между двумя статическими состояниями – от небольшого количества транзиторных микробов в норме до моделей устойчивой микробной колонизации при патологии. Микробиота легких остается малоизученной и загадочной частью патогенеза туберкулезной инфекции.

В настоящем обзоре отражены общие патогенетические механизмы влияния микробиоты легких при респираторной патологии и представлены основные методологические трудности в изучении легочного

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микробиома. Рассмотрены результаты доступных исследований, посвященных изучению особенностей микробного разнообразия легких человека при туберкулезе с применением методов метагеномного секвенирования. Несмотря на высокую вариабельность представленных данных, дисбиоз при туберкулезе чаще характеризуется снижением бактериального разнообразия и обогащением легочной микробиоты анаэробными представителями. *Acinetobacter*, *Campylobacter*, *Moraxella*, *Pseudomonas*, *Staphylococcus* и *Streptococcus*, а также некоторые другие микроорганизмы указываются как важные патогенетические факторы дисбиоза при туберкулезе легких, роль которых еще предстоит выяснить.

Ключевые слова: микробиота, микробиом, легкие, туберкулез, метагеномика

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INTRODUCTION

Modern approaches, including studies based on metagenomic sequencing, have confirmed the existence of lung *microbiota*, a community of microorganisms that colonize lung tissue. It differs from the microbiota of other biotopes of the human body in its low biomass and dynamic diversity of composition because the respiratory tract is a heterogeneous environment with a low nutrient content and high levels of phospholipids, antimicrobial peptides, and immune system cells that prevent microbial colonization [1]. A low bacterial load in the lungs is physiologically maintained to ensure efficient gas exchange.

Lung microbiota, along with the microbiota of the gastrointestinal tract (GIT), significantly contributes to the development of respiratory diseases and can thus be considered a pathogenetic factor. In this case, the balance between microbial immigration and elimination is disrupted, and the composition of the lung microbiota varies depending on the pathology. The most studied changes in lung microbiota are associated with exacerbation of chronic obstructive pulmonary disease (COPD) [2], asthma [3], cystic fibrosis [4], obstructive apnea [5], and pulmonary infections [6]. Less studied are pulmonary dysbiosis in HIV [7] and various forms of lung cancer [8].

Although tuberculosis (TB) remains one of the leading infectious diseases of the lungs, the number of studies on the lung microbiota in TB remains very limited, in contrast to changes in the intestinal, skin, and urogenital microbiomes. Previously, we discovered saprophytic *Bacillus licheniformis* and *Brevibacillus spp.* in the composition of biofilms when inoculating sputum from patients with pulmonary TB [9, 10]. Using DNA sequencing, the microbial diversity of caseous necrosis of surgically excised TB lesions was studied [11]. In addition, domestic research has been devoted to the bacteriological analysis of the bronchial microbiota in patients with TB [12] and the lung microbiota in mice in a model of experimental TB [13].

Currently, next-generation sequencing and metagenomic analysis are the gold standards in the study of infectious lung diseases and human microbiota [14–16]. A systematic search identified 20 original studies that examined the composition and structure of lung microbial diversity in human TB using metagenomic sequencing. The aim of this review was to summarize the pathogenetic mechanisms underlying the influence of respiratory microbiota on lung health and to analyze the available studies on human lung microbiota in the context of pulmonary TB. Special attention is paid to the unique methodological difficulties that arise when analyzing the lung *microbiome* (the genetic component of the microbiota).

LUNG MICROBIOTA AS A PATHOGENETIC FACTOR

Currently, the microbiota is considered to be a factor supporting the immune homeostasis of the lungs and a participant in the pathological process in respiratory diseases. The general pattern of the pathological process in lung diseases is a disruption of the biocenosis of the lung, which is usually unfavorable for the proliferation of bacteria. The

conditions for microbial growth in the lungs radically change due to the influx of nutrient-rich mucus and edema, the establishment of oxygen gradients [17], an increase in the concentration of proinflammatory molecules that promote bacterial growth [18], and disruption of local immune defense mechanisms [19]. The lower respiratory tract (trachea and lungs) is not a homogeneous tissue but consists of regions with varying oxygen levels, pH, temperature, mucus content, and immune cell populations. Pathological changes in the lungs are rarely homogeneous. Much more often, areas of damaged tissue are formed in which local inflammation and changes in microenvironmental conditions occur. In turn, a positive feedback loop is established between microbiota dysbiosis and lung disease. Such changes give a differential advantage in survival to some types of bacteria and impair the growth of others; therefore, the composition of the lung microbiota differs in different diseases [20].

Although the lungs are highly aerated organs, their microbiota contains species with different oxygen requirements. Members of the Bacteroidota (*Prevotella* and *Porphyromonas*) and Fusobacteriota phyla are obligate anaerobes, whereas Bacillota, Pseudomonadota, and Actinomycetota consist of both obligate aerobic (*Pseudomonas* and *Neisseria*) and facultative (*Streptococcus* and *Haemophilus*) and obligate anaerobic (*Veillonella*) genera. A decrease in oxygen levels in the lungs occurs with irreversible airway obstruction when the surface area available for gas exchange decreases by 90 %, which promotes the growth of anaerobic microorganisms [21]. An anaerobic or oxygen-depleted microenvironment that is optimal for the enhanced growth of anaerobic microorganisms is formed in COPD, emphysema, pulmonary fibrosis, and caseous necrosis in a TB focus [22]. During inflammation, the local pH in the lungs can decrease, which promotes the development of acidophilic microorganisms, such as *Lactobacillus* (phylum Bacillota) infection in COPD. The general ratio between Bacteroidota and Bacillota changes in favor of the latter because most Bacteroidota are pH sensitive and cannot develop in an acidic environment [20]. Numerous inflammatory metabolites (catecholamines, inflammatory cytokines), elevated temperature, and free ATP are also growth factors for some bacterial species [21].

Healthy airways produce relatively little protective mucus (about 100 ml per day). To avoid the protective properties of the mucous layer, some microorganisms use specific mechanisms, for example, the formation of biofilms – bacterial populations enclosed in a secreted polymer matrix, attached to each other and the biotic surface [23]. Although the growth of biofilms in healthy lungs remains questionable because of the very low bacterial load and mucociliary clearance, biofilms may have pathogenetic significance in many diseases. In patients with cystic fibrosis, high mucus production and impaired mucociliary clearance are observed in the respiratory tract. Its increased production leads to local foci of anoxia, increased temperature, and promotes persistent bacterial colonization [21]. For example, areas with high mucus levels provide an additional competitive niche for *Pseudomonas aeruginosa* [24] and the nontypeable *Haemophilus influenzae* [25]. This form additionally helps pathogenic bacteria to become more resistant to antibiotics [23]. However, according to our observations, modern clinical strains of *Mycobacterium tuberculosis* (MTB) in most cases do not form biofilms *in vitro* but are capable of producing them in mixed cultures [9]. Drug resistance factors or the host environment surrounding the pathogen may interfere with the production of MTB biofilms [10].

Thus, lung dysbiosis does not always play the role of a leading link in the pathogenesis of respiratory diseases, and cause-and-effect relationships can be multidirectional depending on the pathology. General pathogenetic mechanisms of the influence of microbiota on lung health can be described as an imbalance between immigration and elimination of microorganisms, changes in the composition of the microbiota due to microanatomical differentiation of biotic and abiotic conditions, and the use of specific pathogen-associated mechanisms by microorganisms.

LUNG MICROBIOTA IN TUBERCULOSIS

Despite more than a century of studies on MTB, gaps remain in the understanding of the factors determining the pathogenesis and clinical outcome of pulmonary TB. Multidirectional interactions of immune cells with MTB [26, 27] and probably with lung commensals [28–30] play an important role. A systematic search identified 20 studies aimed at analyzing the characteristics of lung microbiota in humans with TB using metagenomic sequencing methods. More than half of them were published in the previous 3 years and are not included in the latest available reviews [30, 31]. An analysis of the data obtained from these studies is briefly presented in Table. Most studies have been conducted on sputum samples using 16S rRNA gene amplification and

sequencing. Bronchoalveolar lavage (BAL) fluid was used less frequently, as shotgun metagenomic sequencing. Thus, the design of the conducted studies imposes certain limitations on the interpretation of the data obtained. In some cases, the study results are highly variable and contradictory.

The works by Z. Cui et al. [32] and F. Valdez-Palomares et al. [33] showed that active TB is associated with higher taxonomic diversity in lung microbiota. On the contrary, most other studies report a decrease [34–39] or an insignificant change [40–43]. Many studies have found differences in the relative abundance of taxa between patients with TB and controls, sometimes down to the species level. Although it is difficult to compare the general view between different studies, several studies have shown that in TB patients, the microbiota is enriched in members of the phyla Actinomycetota (presumably due to the detection of MTB) [37, 43] and Pseudomonadota [35, 40, 43], the family *Bacillaceae* [36, 44], the genera *Acinetobacter* [39, 44], *Campylobacter* [45, 46], *Moraxella* [33, 44], *Pseudomonas* [34, 39], and the species *Staphylococcus aureus* [38, 47]. Regarding the occurrence of the phyla Bacteroidota [37, 40] and Bacillota [35, 37], the genera *Rothia* [42, 44] and *Streptococcus* [35, 41, 48], controversial results that are directly opposite to each other have been obtained. Many other microorganisms, such as *Cupriavidus*, *Porphyromonas*, *Neisseria*, *Haemophilus*, *Selenomonas*, and *Fusobacterium*, have been considered by various authors to be likely important pathogenetic factors in TB infection.

The enrichment of the pulmonary microbiota with anaerobes (*Prevotella*, *Campylobacter*, *Staphylococcus*, *Streptococcus*, *Selenomonas*, *Fusobacterium*, *Porphyromonas*) and the accumulation of various metabolites of anaerobic fermentation in the lungs observed in TB are associated with changes in pulmonary immunity and the progression of TB infection. Thus, an increase in the proportion of *Prevotella* in the lungs correlates with the concentrations of short-chain fatty acids (propionate and butyrate) [52]. These compounds suppress the production of interferon (IFN)**γ and** interleukin (IL)-17A in response to stimulation of peripheral human blood mononuclear cells by MTB antigens. This immunological effect increases the susceptibility of HIV-infected patients to TB and possibly contributes to the progression of latent TB to active disease. Elevated pulmonary short-chain fatty acid levels are also associated with Treg cell induction [53]. Their population increases significantly in the blood of patients with active TB and suppresses the production of IFNγ [54] and proliferation of MTB-specific effector T cells [55].

Anti-tuberculosis treatment is long-term (at least six months) and includes a combination of narrow- and broad-spectrum anti-tuberculosis drugs. Long-term antibiotic treatment is considered to have deleterious effects on the structure and composition of microbial communities coexisting in the host. Only one of the analyzed studies did not confirm the effect of anti-tuberculosis drugs on the lung microbiota [50]. Other studies have shown a significant decrease in microbial diversity during treatment with anti-tuberculosis drugs [43, 51]. G. Xiao et al. demonstrated a differential effect of treatment on the composition of the lung microbial community with decreased abundances of *S. aureus*, *Pasteurella multocida*, *E. coli*, and *N. gonorrhoeae* and an increase in the number of *Prevotella melaninogenica*, *P. jejuni*, *Ralstonia pickettii*, *Neisseria subflava*, and *Prevotella intermedia* [38].

Some studies have noted low relative abundance of mycobacteria (the percentage of single reads from the total metagenome) [36, 40]. We also obtained a similar result during a pilot study of the microbial diversity of caseous necrosis in several tuberculomas [11]. In addition, Y. Hu et al. showed that there are differences in lung bacterial communities between smear-positive (MTB^+) and smear-negative TB patients (MTB^-) [37]. Such changes in the satellite microbiota of the lung appear to play a significant role in the pathophysiology of TB, which remains to be elucidated.

LIMITATIONS IN LUNG MICROBIOME STUDIES

It must be emphasized that researchers of lung microbiome (both normal and pathological) are faced with methodological difficulties unique to this niche. Almost all studies on lung microbiota have focused on sputum or BAL fluid samples, and there are significant technical difficulties in collecting samples from the lower respiratory tract. Although some studies have generally found minimal contamination of bronchoscopy specimens with upper respiratory tract microbiota [56], others have demonstrated that specimens may be significantly contaminated with nasopharyngeal bacteria [57]. This imposes significant limitations in differentiating the microbiota of the lungs from that of the upper respiratory tract. Invasive methods, such as open lung biopsy, allow one to obtain more reliable material for research [11,

Table

Studies conducted on lung microbiota in human TB

Reference	Sample type and size (TB) / (Control)	Method	Dominant microbiota in TB	Taxa that differ significantly in abundance in TB
Z. Cui et al. [32], 2012	Sputum (31) / Oropharynx samples (24)	16S rRNA	Pseudomonadota (*Phenylobacterium*, *Stenotrophomonas*, *Cupriavidus*, *Pseudomonas*), Actinomycetota, Crenarchaeota	↑ *Sphingomonas*, *Brevundimonas*, *Diaphorobacter*, *Mobilicoccus*, *Brevibacillus*
M. K. Cheung et al. [40], 2013	Sputum (22) / (14)	16S rRNA	Bacillota (*Streptococcus*), Pseudomonadota (*Neisseria*), Bacteroidota (*Prevotella*), Actinomycetota (*Actinomyces*), Fusobacteriota (*Fusobacterium*, *Leptotrichia*)	↑ Pseudomonadota, Bacteroidota, *Mogibacterium, Moryella, Oribacterium*
J. Wu et al. [34], 2013	Sputum (75) / Oropharynx samples (20)	16S rRNA	Bacillota (*Streptococcus*, *Gramulicatella*), Pseudomonadota (*Pseudomonas*)	↑ *Pseudomonas*
L. E. Botero et al. [41], 2014	Nasal, oropharynx, and sputum samples (6) / (6)	16S rRNA	Bacillota, Bacteroidota, Pseudomonadota, Actinomycetota, Fusobacteriota	↑ Streptococcaceae
Y. Zhou et al. [49]P, 2015	BAL (32) / Saliva (24)	16S rRNA	Pseudomonadota (*Cupriavidus*, *Acinetobacter*), Actinomycetota (*Mycobacterium*), Bacteroidota (*Prevotella*, *Porphyromonas*)	↑ *Mycobacterium, Porphyromonas*
P. Krishna et al. [44], 2016	Sputum (25) / (16)	16S rRNA	Pseudomonadota (*Neisseria*), Bacillota (*Streptococcus*, *Veillonella*), Fusobacteriota, Actinomycetota, Bacteroidota	↑ *Corynebacterium*, *Atopobium, Rothia mucilaginosa, Bacillus, Enterococcus, Megasphaera, Veillonella dispar, Lautropia, Acinetobacter, Moraxella*
J. A. Vázquez-Pérez et al. [35], 2020	BAL (6) / (10)	16S rRNA	Bacillota, Pseudomonadota, Bacteroidota, Actinomycetota, Fusobacteriota, Cyanobacteriota	↑ Pseudomonadota, Bacillota ↓↓ *Streptococcus* Only in TB *Lactococcus* and *Leuconostoc* were found
Y. Hu et al. [37], 2020	BAL (6 MTB^{+}-TB) / (6 MTB^{-}-TB)	Shotgun, 16S rRNA	Actinomycetota (*Mycobacterium*, *Rothia*, *Actinomyces*), Bacillota (*Streptococcus*, *Staphylococcus*), Pseudomonadota (*Pseudomonas*), Bacteroidota, Fusobacteriota	↑ Actinomycetota, especially *Mycobacterium* ↓ Bacillota, Bacteroidota
Y. Hu et al. [36], 2020	BAL (12) / –	16S rRNA	Bacillota	↑ *Mycobacterium*, *Bacillaceae*, especially *Anoxybacillus*
C. Sala et al. [50], 2020	Sputum (30) / (30)	16S rRNA	Actinomycetota, Bacteroidota, Bacillota, Fusobacteriota, Pseudomonadota	Not found
E. A. Orlova et al. [11], 2021	Biopsy (12) / –	PCR, 16S rRNA	Bacillota (*Streptococcus*), Actinomycetota (*Mycobacterium*), Pseudomonadota (*Pseudomonas, Brevundimonas*, *Pelomonas*)	Not carried out

D. P. Kateete et al. [51], 2021	Sputum (120) / –	16S rRNA	Bacteroidota (*Prevotella*, *Alloprevotella*, *Porphyromonas*), Bacillota (*Streptococcus*, *Veillonella*, *Gemella*), Pseudomonadota (*Haemophilus*, *Neisseria*), Fusobacteriota (*Fusobacterium*), Actinomycetota (*Rothia*)	Not carried out
M. HaileMariam et al. [42], 2021	Sputum (72) / (54)	16S rRNA	Bacillota (*Streptococcus*), Pseudomonadota (*Haemophilus*), Actinomycetota (*Rothia, Atopobium*)	↑ *Haemophilus* ↓ *Rothia*
F. Valdez-Palomares et al. [33], 2021	Sputum (39) / (6)	16S rRNA	Bacillota (*Streptococcus*, *Veillonella*), Bacteroidota (*Prevotella*), Pseudomonadota (*Neisseria*, *Moraxella*), Fusobacteriota, Actinomycetota	↑ *Ralstonia*, *Moraxella*
L. Ding et al. [47], 2021	BAL (101) / –	Shotgun	Pseudomonadota, Bacillota, Bacteroidota, Actinomycetota	↑ *Staphylococcus aureus*
M. R. Ticlla et al. [46], 2021	Sputum (334) / –	16S rRNA, Shotgun	Bacillota (*Streptococcus*, *Veillonella*), Pseudomonadota (*Neisseria*, *Haemophilus*, *Lautropia*), Bacteroidota (*Prevotella*, *Porphyromonas*), Fusobacteriota (*Fusobacterium*), Actinomycetota (*Rothia*), Saccharibacteria, Spirochaetes, Gracilibacteria, Absconditabacteria, Tenericutes	↑ *Campylobacter*
G. Xiao et al. [38], 2022	BAL (38) / BAL, oropharynx samples (15)	Shotgun	Pseudomonadota (*K. pneumoniae*, *Pasteurella multocida*, *Escherichia coli*, *Neisseria gonorrhoeae*), Bacillota (*S. aureus*)	↑ *S. aureus*, *N. gonorrhoeae*
V. Ueckermann et al. [39], 2022	Sputum, BAL (20) / (51)	16S rRNA	Pseudomonadota (*Burkholderiaceae*, *Enterobacteriaceae*, *Neisseriaceae*), Bacillota (*Lachnospiraceae*, *Veilonellaceae*, *Peptostreptococcaceae*, *Staphylococcaceae*), Actinomycetota (*Micrococcaceae*, *Microbacteriaceae*, *Bifidobacteriaceae*, *Norcardia*, *Actinomycetaceae*), Bacteroidota (*Prevotellaceae*)	↑ *Achromobacter*, *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, *Mycobacterium*
M. Zhang et al. [43], 2022	BAL (23) / (13)	16S rRNA	Bacteroidota, Bacillota, Pseudomonadota, Actinomycetota	↑ Pseudomonadota, Actinomycetota, Acidobacteria, Chloroflexi, AD3
X. Xia et al. [45], 2022	BAL (21) / (57)	16S rRNA	Bacillota, Bacteroidota, Pseudomonadota, Actinomycetota, Fusobacteriota	↑ *Mycobacterium*, *Selenomonas, Lactobacillus, Leptotrichia*, *Campylobacter*

Note: ↑ – taxon abundance increased, ↓ – taxon abundance decreased, ↓↓ – taxon abundance significantly decreased.

58, 59] but are almost always difficult to access. In this way, unaffected areas of the lungs from patients with various forms of cancer obtained during surgical operations were studied as healthy tissue [58], but even these samples can be considered as "conditionally healthy", since patients before surgery received potent immunosuppressive and antibacterial therapy, which can influence the lung microbiota of patients [60].

It is also worth noting that the extremely low bacterial load in healthy lungs leads to a suboptimal signal-to-noise ratio [61], and methods for subtracting the noise component have not yet been standardized and differ between studies. Noise refers to the signal from bacterial DNA, which is the background in endoscopic and surgical sterile equipment, in solutions for nucleic acid extraction, etc. [62]. Specimens with low absolute abundance of bacterial DNA are subject to some degree of stochastic results, named sequencing stochasticity [63].

In shotgun metagenome studies, human DNA is also sequenced; therefore, in respiratory samples with low microbial biomass, the vast majority of sequenced reads are from human genomic DNA [64]. Although 16S rRNA gene sequencing allows for the analysis of taxonomic composition to the species level in some cases [65], the underlying DNA amplification also limits the general view of the microbiome to taxa with inherently high abundances. In addition, the cell walls of some microorganisms (for example, mycobacteria, fungi, capsular forms of bacteria) are resistant to standard DNA extraction techniques [66]. For these reasons, Y. Hu et al. considered that the 16S approach is not the best way to study the TB-associated microbiome, since it is associated with technical limitations in amplification and sequencing of mycobacterial 16S rRNA gene [37].

Lung biogeography – microanatomical differences in microbial diversity – can be a source of bias in studies depending on the method of BAL collection (invasive or noninvasive) and the location of collection. The studies conducted contain controversial results [56, 67]. Based on this, when planning an experiment, careful selection of conditions for all stages of sample preparation of biological material and special caution when interpreting the results are required.

CONCLUSION

Metagenomics is becoming an increasingly actively used approach for studying the characteristics of the pathogenesis and diagnosis of respiratory diseases. Although the study of the lung microbiome was not initially included in the Human Microbiome Project, sufficient evidence has accumulated over the past 10 years indicating the involvement of a dynamic oligobacterial community of the lung in the pathogenesis of TB. However, differences in the study groups, types of clinical samples, and analysis methods still do not allow to definitely conclude about changes in the composition and structure of the lung microbiota during the disease. Each study makes a trade-off between the sensitivity and specificity of the applied methods. More systematic and detailed work, for example, combining metagenomics with *in vitro* cultivation and phenotyping of microorganisms, will likely reveal the complex interactions between MTB and the satellite microbiota of the lung and will also help to determine the specific changes that affect the prognosis of patients with TB.

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