Review Article:
A Review of The Role of The Microbiome on Immune Responses and Its Association With Cystic Fibrosis

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ABSTRACT

In recent years, the microbiome has been recognized as a key regulator of immune responses. Evidence suggests that changes in the microbiome can lead to chronic disease and even exacerbation of the disease. Impairment of innate immunity resulting from microbial incompatibility may worsen host susceptibility to infection and exacerbate chronic lung diseases. Specific microbes play a key role in improving immune responses and microbial incompatibility is involved in chronic lung diseases such as asthma, chronic obstructive pulmonary disease, and Cystic Fibrosis (CF). CF is an extremely complex disease that results from a gene mutation.

Lack of expression of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) has late complications. Incompatibility in lung microbiota is associated with chronic lung diseases, but it is not determined whether this incompatibility can cause health problems or ineffective regulation of immune response create the disease and its progression. In the CF, due to the deficiency of the immune system, many opportunistic microorganisms, including *Pseudomonas. aeruginosa* or *Staphylococcus aureus* are colonized in the patient's lung and due to an immunodeficiency caused by a defect in the system CFTR, lungs are unable to clear the bacteria that leads to severe pulmonary complications and respiratory and digestive problems in such patients. Therefore, in these patients, the microbiome contributes to dysfunctional immune responses and disease exacerbations. This review summarizes the impact of the microbiome on host immune responses and its relationship with CF to explore the role of the microbiome in causing CF.

Introduction

Microbiome is defined as a collection of bacteria, fungi, algae, viruses, protozoa, and microscopic organisms, their genetic elements, and their relationship to the environment. Environmental factors play an important role in microbiome acquisition [1-3].

The type and composition of the microbiota are determined by many factors, including host genetics, age, health or disease, environmental factors, and host immunity. The presence of symbiotic microbes in the

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body and their participation in human physiology has been known for many years, but scientific understanding of the clinical importance of these interactions has expanded in recent years with the development of omics technologies. In general, an adult's body consists up of 10^{14} microbial cells and 10^{13} cells. These numbers indicate that a person's body contains more microorganisms than the individual's cells, suggesting the importance of microorganisms to the body [2, 4-6].

In recent decades, non-culture-based sequencing technology, especially the next generation sequencing, has been developed to understand the relationship between microbiome and disease. In a healthy individual, the body's microbiota consists of six major phyla bacteria: *Firmicutes*, *Actinobacteria*, *Fusobacteria*, Cyanobacteria, *Bacteroidetes*, and *Proteobacteria* [5, 6].

16S rRNA gene sequencing indicates that *Firmicutes* and *Bacteroidetes* make up 92% of the body's microbiome. Although the gut microbiome can contain 1000 to 1500 bacterial species, a person may contain only 160 bacterial species. This difference represents the significant fact that the microbiome content varies from person to person and is affected by the environment and heredity [7].

Respiratory microbiome and infection

Learning more about the respiratory microbiome is an important basis for treating, managing, or preventing respiratory diseases. Disorders in the microbiome population of the respiratory tract, on the one hand, cause immune dysregulation and on the other hand, increase the susceptibility to infectious respiratory diseases. The upper respiratory tract is occupied by a large number of anaerobic bacteria. The predominant bacteria in the respiratory system in healthy people include *Pseudomonas aeruginosa*, *Fusobacteria*, *Prevotella*, *Veillonella*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. The initial part of the throat is surrounded first by *Staphylococcus* or *Corynebacterium* and down there by Moraxella or *Alloiococcus* and then by *Streptococcus* or *Haemophilus*. Monocytes, macrophages, dendrites cells, $CD4^{+}$, and internal lymphoid cells are present in the upper respiratory tract and prevent the establishment of important microbial populations in the lower airways [8, 9]. The importance of airway colonization to balance local immunity has been the subject of many studies, and airway disorders are associated with the spread of disease and infection. For example, infections of the lower respiratory tract in the first few years of life increase the susceptibility to allergic reactions and chronic wheezing in later years. However, the microbiome can reduce the chances of getting diseases and protect the body against bacteria. Examples of the association between microbiome and disease include the regulation of the upper respiratory tract microbiome by *Lactobacillus rhamnosus* or *lactococcus lactis*. On the one hand, they protect the body against the respiratory syncytial virus, and on the other hand, increase the ability to clear *S. pneumonia* from the lungs [8].

Mechanism of the immune response to pathogens

Immunity refers to the tissues, cells, and molecules that protect the body against infection. The immune system includes both innate and acquired immunity. The gastrointestinal tract has the largest and most diverse population of microorganisms as well as a large volume of lymphoid tissue. In the body, the gut-associated lymphoid tissue is the first line of defense in the intestinal mucosa. The immune system detects and responds to allergens in microbial populations. These materials are highly protected. Microorganisms and their immunological compounds, including nucleic acid, cell wall fragments, and flagella are identified via Pattern Recognition Receptors (PRRs), including Toll-Like Receptor (TLR), C-Type Lectin Receptor (CLR), retinoic acid-inducible gene-I-like receptors (RLRs), NOD-like receptors, and CD14+ receptors for bacterial lipopolysaccharides [10-12].

Aerial epithelial cells, macrophages, and dendritic cells identify Pathogen-Associated Molecular Patterns (PAMPs) or Microbe-Associated Molecular Patterns (MAMPs). PAMPs include cell wall lipoprotein, lipopolysaccharide, and flagella, and MAMPs contain microbial cell surface molecules such as lipopolysaccharide, teichoic acids, peptidoglycan, extracellular polysaccharide, flagella, DNA, and dsRNA [13-15]. These interactions lead to the production of immune mediators through myD88 or apoptosis-related proteins that create cascading pathways and produce pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-a), interleukin-8 (IL-8), and IL-1B [16]. Detection of pathogens and the release of inflammatory mediators are used to organize neutrophils and rapidly detect and clear pathogens [2, 11, 17, 18]. These compounds act as immune-stimulating factors. One of the functions of the immune system is to distinguish between the microbiome and the pathogen. As the body's first line of defense, the innate immune system must be able to detect the type of microbe, which includes the identification of specific patterns unique to the microbe. One class of molecules that recognize such patterns is the family of pattern-recognizing receptors, which include TLRs. These are the

main innate immune receptors for identifying and sensing bacteria [10, 19].

Host defense mechanism in the respiratory epithelium

The first line of defense against pathogens is the innate immune system, which activates immune responses associated with the activation of T and B cells against specific antigens. The innate immune system not only provides a primary barrier against colonization and infection but also determines which antigen triggers an immune response. In the airway, the host's innate defense system works with several other mechanisms to protect the airway and lungs from colonization and infection [20].

Mucosal clearance provides an effective system for clearing pathogen particles and is associated with aerodynamic filtering and air reflex reactions such as sneezing and coughing. The mucus is produced by the mucous glands and goblet cells and then pushed forward by the movement of the lashes. The cells of the innate defense system which include macrophages, neutrophils, and epithelial cells not only act effectively against pathogens but also activate host responses to infection. Airway secretions include proteins and peptides which either directly kill pathogens, prevent them from entering into the lungs, or balance inflammatory responses. These chemicals are produced by secretory systems in the respiratory system such as glands, goblet cells, Clara cells, and type II pneumocytes in the lung parenchyma. Major constituents of airway surface fluid that have antimicrobial activity, including lysozyme, lactoferrin, secretory phospholipase A2, and secretory leukocyte protease inhibitor [13].

Other compounds such as complement or surface Clara-cell specific protein have a partnership role. In addition to the production of mucus by goblet cells, all airway epithelial cells can produce antimicrobial peptides that play an important role in limiting the presentation of commensal microbiota. This protein can display antimicrobial activity that binds enzymatically to the bacterial cell wall or destroys the bacterial inner membrane. The mucus restricts the communication between the microbiota and the host tissue and prevents movement of the microbes. Antimicrobial peptides are key players in host defense at mucosal levels. Peptide antibodies found in plants and animals are made as preproteins, released after the division of mature peptides, and activated through microbial permeabilization. One of these peptide antibiotics is defensins. They are cysteine-rich peptides with high antimicrobial activity that are expressed by epithelial cells and many other cells [20].

Induction of a protective response by microbiomes

Research has shown that sensing common microorganisms plays an important role in homeostasis. Some bacteria produce compounds such as polysaccharides produced by the *Bacteroides fragilis*, which can protect mice from *Helicobacter hepaticus*, a pathogenic bacterium. This protection is related to its capsular polysaccharide A ability to induce the production of IL-10-producing Treg-cell. *Bacteroids* can improve Treg-cell function by increasing TLR2 expression. This association between specific microbiota and immune cell induction limits mucosal infections and promotes tolerance. The protection of the host against pathogens by commensal bacteria indicates resistance to colonization. One of the forms of communication between microbiota and invasive bacteria is a common ecological niche. Microbiota limits the colonization of pathogens. Change in access to food by microbiota can affect the expression of virulence genes and the growth of pathogens such as *Escherichia coli* and *Clostridium* [12].

The immune effects of microbiota in the body depend on the specific location. For example, colonized *Staphylococcus epidermidis* on the skin stimulates the production of interferon-gamma (IFN-γ) from $CD4^+$ which protects the body against the pathogen *Leishmania major*, whereas intestinal colonization with *Staphylococcus epidermidis* does not have this protective effect. It has been shown that changes in the gut microbiome can also affect immune responses in more distant places.

Antibiotic treatment that disrupts the intestinal microbiome and causes fungal colonization can increase allergic responses to *Aspergillus fumigatus* spores in the nasal passages. Alternatively, balancing the intestinal microbiome with probiotics increases the secretion of IgA from B cells in the colon and lymph nodes and increases the T follicular helper in the lymph nodes and dendrites of IL-23 secreting cells. The combination of these changes improves host defense at the mucosal site and response to vaccination [21, 22].

Commensals can change environmental conditions, such as the vaginal environment, for example, *Lactobacillus* can inhibit pathogen colonization by lowering the pH. Commensals cause innate immune cells to respond rapidly to pathogens: a controlling role in the gut microbiota to control IL-1B production [23, 24].

All these events demonstrate the role of microbiota in shaping, activating tissue Treg-cell, and maintaining

host-microbial balance at barrier sites. The microbial pressure of the lungs induces and maintains the activity of Treg-cells, which plays a role not only in maintaining mutual communication with the microbiota but also in the systemic control of the immune response [12].

Lung microbiome and its relationship with the intestinal microbiome

Culture-based methods showed that healthy people had no bacteria in their lungs, and this theory had been widely accepted for many years. About 20 years ago, non-culture-based methods based on 16S rRNA analysis were used to study environmental microorganisms and the human microbiome. These studies showed that the lungs were not sterile but colonized by a wide range of microbiota [16]. The composition of the lung microbiota is similar to that of the respiratory microbiota, but they are fewer in number and include the following bacteria: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *P. aeruginosa*, *Veillonella*, and *Prevotella*.

Lung bacteria settle in the lungs during the first two weeks of a person's life and change from *GammaProteobacteria* and *Firmicutes* to *Bacteroidetes*. This change in the microbiota is associated with a population of Treg cells that cause allergen tolerance. These microbiotas are important in the early years of life to protect the lungs from destructive responses to respiratory antigens [5, 6].

PRRs sensitize microbial compounds and induce differentiation of Treg and Th17 cells. PRR in the lungs differentiates microbial compounds from microbiota and changes immature T-cells to Th1, not Th2. Before birth, the pattern of the immune system is determined by Th2, but after birth, immature T cells in the lungs change from Th2 to Th1, thus protecting the body against allergic diseases [25]. Ichinohe found that intestinal microbiota played a key role in the development of viral $CD4^+$, $CD8^+$, and antibody responses after infection with the respiratory influenza virus. Chen found that the destruction of intestinal microbiota by antibiotics increased the number of bacteria in the blood and lung, and even in a study, *Escherichia coli*-infected mice died due to the lack of intestinal microbiota [7, 26]. Further studies show no influenza virus in the intestinal tissues after nasal infection, and the influenza virus may have caused infection and impaired intestinal immunity. Decreased diversity in the gut microbiome as a result of viral infections or overuse of antibiotics leads to poor interleukin-17 (IL-17) function or bacterial killing mechanisms, which can lead to overgrowth and enlargement of pathogen-infected organs, eventually disease exacerbation. Some types of chronic lung diseases may be due to the movement or spread of gut bacteria. Pulmonary problems caused by a viral infection or other factors can exacerbate the disease by affecting the gut or lung microbiota. For example, changes in systemic cytokines such as Th2 and Th17 can cause bacterial fibrosis [7, 27].

Compared with the intestines, the lungs have fewer nutrients to support the microbiome. Physiological conditions vary even in the lungs of healthy people. Factors that affect bacterial overgrowth include oxygen pressure, blood flow, pH, temperature, epithelial cell structure, and condition of cells affected by the infection. The basic strategy by which the host-microbiota relationship is kept balanced to limit the contact between microorganisms and the surface layers of the epithelial cell. Under these conditions, tissue infection is reduced and microbial transport is minimized, which occurs especially in the gastrointestinal tract. Lung microbiota is involved in causing lung disease, and changes in the lung microbiota increase the risk of developing a wide range of diseases. A variety of factors, including anatomical damage, pathological effects, physiological changes, and the immune system can destroy the lung microbiota and lead to chronic diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis (CF), and idiopathic pulmonary fibrosis [5, 6, 28].

The relationship between microbiomes and immune responses in Cystic Fibrosis (CF)

CF is a chronic pulmonary and genetic disease with an autosomal recessive inheritance that is first identified in the North Caucasus. Today, about 30000 children and adolescents in the United States have this disease [29]. So far, about 1200 CF patients have been identified in Iran and this number is increasing every year. CF occurs due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene at locus q31.2 on chromosome 7 [30]. CFTR is a chloride channel that regulates other ion channels, such as bicarbonate, glutathione, and thiocyanate, and can transmit these molecules [15, 31, 32].

In 90% of the patients with CF in the United States, a mutation in the F508del occurs, a mutation in phenylalanine that causes the protein to fold incorrectly, causing the proteasomes to break down and depleting the apical epithelium of this transmitter. Loss of CFTR function has many effects on human health and causes diseases in the pancreas, liver, lungs, stomach, intestines, as well as problems in the male reproductive system. Pulmonary CF disease is characterized by high levels of invading

cells such as neutrophils, lymphocytes, and macrophages, elevated infectious cytokines, and chronic colonization by opportunistic pathogens [19, 33].

These microorganisms create an ecological niche in the lungs of patients to resist inflammatory responses, resulting in structural damage to the airways and reduced lung function. Lung diseases cause limitations in the quantity and quality of life for CF patients [3, 34]. Bacterial pathogens of the CF airway are normal at birth but are soon replaced by opportunistic pathogens [35]. The number of pathogens associated with CF has up-dated with the introduction of non-culture-dependent methods, DNA-based methods, and next-generation sequencing methods [15]. These bacteria which include *Staphylococcus aureus*, *Haemophilus* influenza as well as Gram-negative bacteria of *Klebsiella pneumonia* and *Escherichia coli*, infect children in the first and second year of life, and the lungs of adolescents and adults gradually become infected with *P. aeruginosa*, which changes to *Aspergillus* or *Candida* as the patient gets older [1, 20, 36-39]. The role of these bacteria in the spread of lung disease is unknown, but what is obvious is that *P. aeruginosa* is the predominant infectious bacterium. The infection may be chronic and last for years. Some CF pathogens, including P. *aeruginosa*, Australian epidemic strain, *Burkholderia cepacia*, and *Mycobacterium abscessus* may travel between individuals [1, 39].

CF patients have a mixed microbial population in both the airway and intestine and changes in these microbial populations affect disease progression and clinical symptoms. Alterations, inconsistencies, and the microbial status of the lungs increase mucus secretion and inefficiency in clearing the airways that may cause chronic lung damage. This change may lead to chronic colonization of pathogenic bacteria, which impairs immunity in CF patients and impairs the function of defense barriers [39].

The role of mutations in CF disease

Although immunodeficiency has been diagnosed in CF since 1970, several studies have shown the role of CFTR in the process. Deficiency in CFTR function disrupts signaling pathways as well as the balance and concentration of intracellular ions, including calcium, which are involved in gene expression pathways [37].

Studies have shown that CFTR acts as a receptor for P. *aeruginosa* binding and ion channels are involved in the clearance of bacteria through adsorption to the surface of epithelial cells. On the other hand, CFTR degradation has been shown to increase salt concentrations and inactivate antimicrobial compounds such as defensins [40, 41].

Mutations in CFTR thickens and dries mucus, making it an ideal environment for P. *aeruginosa*. This *bacterium* can switch between mucosal and non-mucosal strains and thus show resistance to phagocytosis. Patients are at risk for chronic infections with opportunistic bacteria such as *P. aeruginosa*. Altered response to *P. aeruginosa* and *Aspergillus fumigatus* is common with increased neutrophil uptake in CF [42].

The immune system responds to these pathogens by releasing neutrophils, which results in the release of infection into the lungs of CF patients. The effect of the CFTR mutation on the immune system may exacerbate the disease by spreading allergic reactions and preventing the pathogens from clearing the patients' lungs. CFTR is an unknown feature of chronic lung infections in CF. Large numbers of neutrophils are present in the lungs of children and adolescents, even in the absence of pathogens, and cause the proteolytic breakdown of elastin and destruction of lung structure [33, 43, 44].

Defect in CFTR in immune cells rather than in the alveolar epithelial cells (AECs) could be the source of lung infections and be responsible for the inability to clear *P. aeruginosa* in mice. Of course, these results cannot be attributed to humans because most studies on *P. aeruginosa* are non-mucoid while clinical isolates constitute biofilms. AECs play a key role in the pathology of CF and indicate a link between the pathogen and the immune system. CFTR-defective AECs exposed to *P. aeruginosa* show higher nuclear factor kappa-B (NF-κB) activity and an increase in IL-8 expression. IL-8 is a highly active neutrophil and its overexpression is seen by AEC in severe CF infections [45].

CFTR maintains homeostasis for the expression of 843 genes. Most of these genes are involved in phagocytosis, apoptosis, DNA replication, T-cell selection, B-cell activation, and chemotaxis. Research has shown that the primary defect in CFTR in innate immune cells is associated with CF pathogenicity. Healthy mice show more severe lung infections when exposed to neutrophils without CFTR. This infection is due to increased transcription of NF-κB [46].

Because neutrophils are the first line of defense against *P. aeruginosa*, their proper functioning is essential in CF patients. According to Morris research, neutrophils in CF patients have a weak phagocytic capacity. The transforming growth factor-beta (TGF-B) signaling pathway is activated to regulate some of these cytokines. An important part of TGF-B is Smad3, a transcription factor that enters into the nucleus and is responsible for binding to promoter

regions of regulatory genes. The safety targets of Smad3 include IL-10, anti-inflammatory cytokines, and myristoylated alanine-rich c-kinase substrate (MARCKS). MARCKS is a protein responsible for rearranging actin to aid in neutrophil migration. Smad3 can regulate infection and anti-inflammatory responses, and increasing Smad3 expression is essential for anti-inflammatory cytokines. IL-10 can be expressed as the anti-inflammatory target of Smad3 to prevent additional inflammatory reactions by resting the immune system [19].

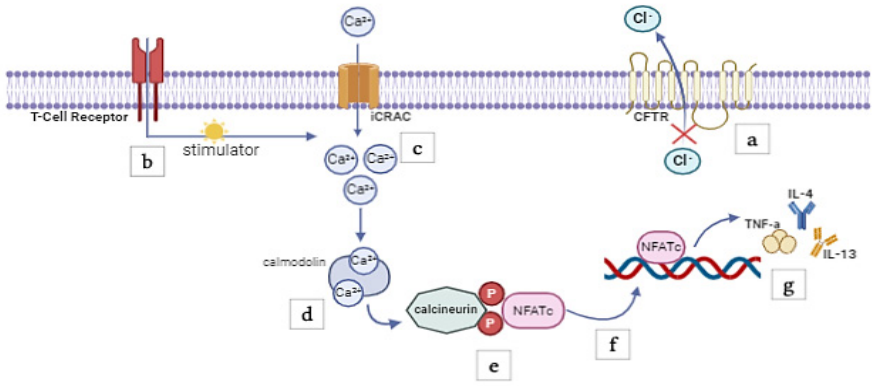
CFTR is expressed in T-cells, and mismatch in these cells may affect immune function. The inefficiency of Th1 responses leads to activation of Th2 allergy responses and an increase in IL-3 and IL-4 and ultimately the production of IgE antibodies by the B cells. IgE causes allergies and infections, and mast cells without CFTR produce a large amount of IL-6. These additional responses damage lung tissues because CFTR-free epithelial cells produce small amounts of glutathione and increase pro-inflammatory prostaglandins. Mutated CFTR induces calcium accumulation, thereby increasing macrophage gene expression. Calcium flux is essential for T-cell activation and is regulated by several ion channels. Disruption of this balance causes abnormal expression of many genes in T-cell, including those responsible for Th1/Th2 differentiation (Figure 1) [33].

According to Figure 1, if intracellular calcium levels rise in CF mice and T-cells under the influence of receptor stimulation, the transfer of nuclear factor of activated T-cells (NFAT) transcription factors to the nucleus increases. NFAT acts as a stimulant of Th2 expression, which includes IL-4, IL-6, IL-13, and ultimately IgE synthesis and infection [33].

Shenoy et al. showed that intracellular calcium levels in wild-type monocytes were increased in the presence of CFTR, ATPase, calcium inhibitors, and this event shows how macrophage gene expression is affected [47]. Increased intracellular calcium levels can alter the expression of many genes in CF. Evidenced by using AEC and neutrophil studies, CFTR affects a wide range of cellular processes. Moreover, there is a mismatch in gene expression in CF monocytes that may affect the ability to clear pathogens [47]. Zamon et al. found that the mitogen-activated protein kinase/ extracellular signal-regulated kinases (MAPK/Erk) pathway was highly sensitive to lipopolysaccharide stimulation and was even present in CF monocytes [48]. MAPKs are involved in many cellular functions such as cell division, apoptosis, and cytokine synthesis. The researchers found that monocytes in CF-producing individuals produced 100 times less IL-8 in response to stimulation of lipopolysaccharides than controls, indicating a reduction in neutrophil utilization at the site of infection with Gram-positive bacteria. Increased activity of macrophage inhibitors in CF individuals also leads to ineffective responses to pathogens [48].

The role of airways and lungs in exacerbating CF

The CF airway is a suitable environment for microbial colonization. CF disease is expressed by a decrease in the amount of Airway Surface Fluid (ASF), an increase in mucus viscosity, and a loss of the ability to remove mucus by hairy cells. Thus, the primary defense mechanism is disrupted and a healthy environment is provided for microbial growth. In addition to amino acids, iron, lactoferrin, and ferritin are present in large amounts in the patient's sputum and lung tissues and play a role in increasing the severity of the disease [39].



IMMUNOREGULATION

Figure 1. The lack of CFTR function and upregulation of T cell polarization increase calcium uptake

Through the high activity of T-cell receptors and the opening of calcium channels and finally leads to increased expression of pro-inflammatory cytokines, which are characterized by Th2 immune responses.

Iron levels are positively associated with infectious cytokines, IL-1B, TNF-a, and ferritin, but are negatively associated with lung function. In patients positive for *P. aeruginosa*, iron levels are positively correlated with bacterial levels. Iron-rich nutrients lead to microbial growth and chronic infection. Mucus in the CF airway is deoxygenated and some microorganisms, including *P. aeruginosa*, can grow under anaerobic conditions [39].

Low oxygen or anaerobic conditions allow *P. aeruginosa* to access additional environmental niches. Host defense molecules play an important role in clearing the mucosa as a key barrier in the lungs and contain a wide range of antimicrobials. However, changes in airway balance lead to the destruction of these defense barriers, which may be due to changes in pH, protein oxidation, and protease activity. For example, in a study of pigs with CF with the F508-CFTR mutation, a decrease in pH in ASF occurred as a result of a defect in the function of antimicrobial compounds such as lactoferrin and lysozyme, which ultimately led to a defect in killing bacteria. The poor efficacy of B-defensin is associated with abnormal ASF inactivation [39].

On the other hand, anaerobic or low air conditions in the respiratory epithelium in CF cause mucoid strains and become resistant to phagocytosis. It also alters the structure of the outer membrane during the anaerobic growth of *P. aeruginosa*, leading to the development of resistance to cationic antibiotics such as aminoglycosides [39].

The role of immunity in CF disease

One of the major symptoms of CF lung disease is a chronic state of infection, and there is much debate about the causes of this condition. An important aspect of CF is the change in acquired immune responses to specific pathogens. There is evidence that the immune response in CF is impaired, leading to a defect in the response to chronic infections. CF patients tend to have allergic reactions such as asthma, skin inflammation, and pro-inflammatory immune responses. Lymphocytes may not be directly but indirectly involved in acquired immune responses. Failure to regulate secretions may reduce the response to antigens and the inability to clear *P. aeruginosa* infections that impairs pulmonary function. Microorganisms present in the airway of CF individuals can easily escape the immune response or can take advantage of the host immune responses [43, 49].

In recent decades, in vitro experiments have shown that chromosome signaling can balance the production of pro-inflammatory cytokines, a process that occurs in lymphocytes and leads to neutrophil and macrophage apoptosis. Chromosome signaling molecules also react with eukaryotic target proteins and alter gene expression in mammalian cells [50].

Targeting bacterial DNA may activate neutrophils through a TLR-independent mechanism that increases the expression of intracellular signaling pathways and the production of IL-8. TLR2 and TLR4 are involved in activating monocytes through mannuronic acid compounds produced by *P. aeruginosa* [51]. Besides, *P. aeruginosa* activates quorum sensing through the production of rhamnolipids, a bacterial product that induces rapid neutrophil necrosis. The regulation of rhamnolipid synthesis is controlled by quorum sensing *P. aeruginosa*. Then, it uses the necrotic neutrophil content to develop a biofilm, which involves the integration of neutrophil-derived actin and DNA into the biofilm, allowing other bacteria to be added to the biofilm [10, 43, 52, 53].

TLR5 is the only TLR that is elevated in neutrophils in people with chronic CF infection. This increase in expression may be due to IL-8, TNF-α, granulocyte colony-stimulating factor, or in association with TLR1 and TLR2 as a result of binding to bacterial lipopolysaccharide. TLR5-dependent phagocytosis may enhance host defense against planktonic and flagellate populations of *P. aeruginosa* in CF. Lymphocyte dysfunction in CF against *P. aeruginosa* has been known before the 1970s. T cells in CF appear to tend to Th2, which is a pro-allergy factor and is suitable for defense against parasites but is not effective for encountering *P. aeruginosa* [16].

Laboratory data show that 10% of CF patients show allergic reactions in the lungs and bronchi due to *Aspergillus fumigatus*, in which IL-4, IL-13, and IgE are increased. This compound, IgE, appears to be a pre-inflammatory immune response due to Th2. High levels of IgE are seen in mice exposed to *Aspergillus fumigatus*, which is associated with a clear shift in cytokines IL-3 and IL-4 and increased sensitivity of CF patients' B cells to IL-4 stimulation [33, 54]. This action is caused by a disorder in CFTR in immunocompromised mice. These results indicate the role of defective CFTR lymphocytes in causing allergic inflammation in CF. Quorum sensing signaling in *P. aeruginosa* reduces IL-10 production without altering its release [19, 35]. Additionally, an increase in IL-8, IL-6, IL-1B, IL-17, and TNF-a can be seen in CF patients. Dendritic cells are exposed to chromosome signaling, a process that occurs during antigen stimulation and reduces the ability to induce specific T-cells. Quorum sensing signaling molecules in *P. aeruginosa* prevent dendritic cells from exerting their

T-cell stimulatory effects. These act as immunomodulators and cause *P. aeruginosa* infections in the host. In CF patients, many of these processes are damaged. For example, bronchial epithelial cells present ectopic PRRs, leading to a steady increase in NF-κB activity. Saliva and bronchoalveolar lavage increase the levels of pro-inflammatory cytokines such as TNF-a, IL-6, IL-8, IL-1B, and a decrease in IL-10 [43, 49, 53]. Some of these responses against bacteria are presented in Table 1.

The role of the microbiome in CF disease

CF was associated with early colonization of *Staphylococcus aureus* in the lung and some cases with *Haemophilus* influenza. However, the survival of H. influenza was not certain. Evidence shows that *Staphylococcus aureus* causes severe lung disease. The primary pathogens that lead to infection and damage the airways appear to act as mediating organisms, providing a pathway for the colonization of *P. aeruginosa*, the main pathogen in CF. Evidence suggests that *P. aeruginosa* may have been the primary source of environmental reservoirs, although cross-infection has also been reported. Colonization with *P. aeruginosa* is an important factor in the progression of CF, especially if it was associated with *Staphylococcus aureus*, all of which are associated with virulence factors that are resistant to antibiotic therapy and the immune response [51].

P. aeruginosa causes the expression of virulence factors and can escape from the host immune system. There is evidence that *P. aeruginosa* adapts to the lungs of CF patients. There are many substances, such as various amino acids, in the airways of CF patients that are highly needed for *P. aeruginosa*, and the bacteria use these substances readily. On the other hand, many adaptations in bacteria have led to the gradual elimination of virulence factors necessary for acute infections, so it is not uncommon for P.aeruginosa isolated from CF to have less virulence than other P.aeruginosa [32, 58].

This reduction in virulence helps the bacteria to escape the host immune response. This process takes place by limiting the factors known to the host, and these factors may stimulate inflammatory responses and lead to less tissue damage. On the other hand, strains that are not able to produce virulence factors can grow rapidly. During chronic colonization in the CF lung, *P. aeruginosa* clones are affected by variability in morphotype, motility, virulence, antimicrobial susceptibility, and production of adhesion, exopolymeric substances, and secondary metabolites. In addition to detecting lipopolysaccharide via TLR4, *P. aeruginosa* is detected by the host through a flagellum sensation via TLR5 and that is important for killing bacteria by macrophages. Loss of movement is common in chronic infectious strains. The change in the expression of flagellum occurs in response to CF mucosal compounds which cause it to escape host and phagocyte detection. Flagellin induces the accumulation of Myeloid-Derived Suppressor Cells (MDSC), which has been shown to occur in CF patients who encounter *P. aeruginosa* for the first time. MDSC can escape acquired immunity by blocking T-cell responses [58].

Surviving mice become resistant to re-infect with *P. aeruginosa* and spread this resistance. Besides, CF mice

Table 1. Summary of immune responses in CF patients caused by bacteria

Bacteria	Immune Response	Reference
Pseudomonas aeruginosa	Increase in IL-1, IL-6, IL -18, IL-12, TNF-α, IL-8, ICAM-1 Decrease in IL-10	[19]
Pseudomonas aeruginosa	Increase in TNF-α, IL-6, IL-8, IL-1β, IL-17, IL-23 Decrease in IL-10	[15]
Pseudomonas aeruginosa	Increase in IL-6, TNF-α, IL-8, IL-1β Decrease in IL-10	[41]
Pseudomonas aeruginosa	Increase in IL-4, IL-13, IL-8, IL-1β, IL-6, TNF-a Decrease in IL-10, IFN-γ	[55]
Pseudomonas aeruginosa	Increase in IL-8, Th2, IL-4, IL-13, IgE, IL-6 Decrease in Th1	[33]
Aspergillus fumigatus	Increase in, IL-4, IL-13, IgE, IL-6	[33]
Aspergillus fumigatus	Increase in IL-5, IL-13, IFN-γ, IgE	[56]
Aspergillus fumigatus	Increase in IL-13,IL-4, IgE	[54]
Aspergillus fumigatus	Increase in IL-4, IL-5, IL-10, IgE	[57]

IMMUNOREGULATION

Interleukin: IL; tumor necrosis factor-alpha; TNF-α: interferon-gamma, IFN-γ: intercellular adhesion molecule-1, ICAM-1.

colonized with *P. aeruginosa* have high antibody titers against *P. aeruginosa* antigens but are still susceptible to chronic infection. Thus, the Th1-dependent immune response has a protective role against *P. aeruginosa* infection and is not dependent on antibody production. Another mechanism that enables *P. aeruginosa* bacteria to remain chronically in CF patients and lead to disease is the killing of neutrophils by *P. aeruginosa* through protease secretion which not only kills neutrophils adjacent to the *bacterium* but also affects neutrophils at other places. Thus, the *bacterium* survives and can cause more infection, more tissue damage, and eventually create a suitable environment for chronic infection. Hartl examined molecular changes in neutrophil structures and showed how they might lead to defects in the treatment of the disease [59].

Concerning other works, these new findings on the biology of neutrophils are acceptable not only in the case of CF but also in the case of other diseases and conditions in which neutrophils play a major role. Mucosal immunosuppression allows infections to settle in patients' lungs. Neutrophils are activated by the production of cytokines such as IL-8, which are secreted by various cells such as macrophages and epithelial cells. Neutrophils express two IL-8-related receptors: CXCR1 (chemokine (C-X-C motif) receptor 1) and CXCR2 (chemokine (C-X-C motif) receptor 2). Both receptors bind to IL-8 with the same affinity. But there is a specific function for each. CXCR2 appears to mediate the initial utilization of neutrophils after the receptor loses its signaling ability and its expression decreases rapidly [60].

CXCR1 is more resistant to loss of sensitivity and is likely to help transport neutrophils to areas with high levels of IL-8. CXCR1 is involved in the development of antimicrobial responses. Hartl found that IL-8 signaling via CXCR1 was important for the effective killing of *P. aeruginosa* and those neutrophils present in the airway of CF patients with low levels of CXCR1 and defense markers in killing bacteria [59].

Hartl showed that neutrophil proteases, elastase, and cathepsin G lead to the cleavage of CXCR1 in living neutrophils and inhibit their bactericidal activity [59]. However, bacterial protease such as *P. aeruginosa* elastase, which is known to disrupt other components of the immune system, may have unknown effects. CXCR1 signaling may mediate many anti-apoptotic activities of IL-8 at the neutrophil level. Therefore, *P. aeruginosa* can kill neutrophils and create an infectious environment capable of altering neutrophil proteases against friends, disabling neutrophils from killing bacteria, and disrupting neutrophil responses to host survival symptoms. Hartl found that CXCR1 fragments help produce IL-8 through activating TLR signaling in bronchial epithelial cells. This was the first time that split chemokine receptors could activate TLR. However, the use of neutrophils in CF may limit the spread and pathogenicity of *P. aeruginosa* but provide a dysfunctional infectious environment, allowing *P. aeruginosa* to use necrotic neutrophil secretory compounds such as DNA and actin remains stable in the biofilm and contact with other bacteria. To identify the clinical effects of this new finding, Hartl showed that treating CF patients with anti-proteases increased neutrophil CXCR1 expression in the airway and improved the ability of these cells to kill bacteria. Targeting this receptor may raise risks. CXCR1 can reduce neutrophil activation at the site of infection but may cause a defect in killing bacteria at the site of infection [59]. Staphylococci also change their phenotype under anaerobic conditions to form polysacharidenased cells and resist non-oxidative killing. Burkholderia strains that are resistant to non-oxidants cause CF disease. Because the anaerobic environment is enclosed in the lung epithelium, bacteria are not seen in patients and chronic microbial infections occur. As a result, bacteria that can form biofilms or are resistant to non-oxidative killing can colonize the lungs of CF patients [60].

Airway microbiota alters the response to a variety of factors, most notably the host immune response and treatment, and ultimately the diversity and specificity of the microbiota vary from person to person, depending on age, decreased lung function, and disease progression. How the microbiome regulates innate immunity in patients and healthy individuals has been the subject of debate in the scientific community, and tiny is thought about how the lung microbiota regulates pulmonary immunity or the event of bronchial-associated lymphatic tissue. In the absence of normal gut microbiota, the host is more susceptible to opportunistic infections such as *Listeria*, *Klebsiella*, and even viruses. Chronic lung disease may occur as a result of defective innate and acquired immunity and as a result of changes in the gut microbiota. For example, in the case of idiopathic pulmonary fibrosis, there is evidence that Staphylococcus and *Streptococcus* are high in patients' lungs and the ability of mouse neutrophils to kill these bacteria is reduced [7].

Treatment options that target the host and pathogens response

High levels of ibuprofen reduce neutrophil migration and reduce the progression of CF lung disease, especial-

ly in children. The mechanism of action of ibuprofen is unknown, but it is probably due to a reduction in CD11 misalignment and actin polymerization in response to IL-8 and C5a [61].

Azithromycin improves lung function in CF patients and reduces pulmonary responses by inhibiting cytokine production, increasing tight junction, and protecting the respiratory epithelium from injury [62, 63]. N-acetyl cysteine breaks disulfide bonds to prevent biofilm formation, to polymerize mucus, and to prevent the formation of neutrophil extracellular traps [64].

Corticosteroids are potent anti-inflammatory agents and reduce neutrophil adhesion and epithelial transmission, but the steroid-dependent side effects limit the use of this drug [65]. Leukotrienes are also abundantly produced in the CF airway. Its inhibitor, which is very common in the treatment of asthma, is not used in the treatment of CF, which is probably due to the non-specific effect of the drug on leukotriene [66].

There are many goals for treating CF infections. Neutrophils produce large amounts of elastase that are normally neutralized by alpha-antitrypsin (AAT). AAT reduces airway disease in mice with *P. aeruginosa*. Recombinant human AAT in CF patients is being tested [67].

KB001-A is a humanized monoclonal Fab fragment that targets the *P. aeruginosa* virulence factor (Type III secretion system). Phase 2 treatment in non-CF patients treated with *P. aeruginosa* was safe and showed a decrease in *P. aeruginosa* colonization in patients. The results of the experiments show a decrease in IL-7, IL-8, elastase, and neutrophils in CF patients who received single doses of KB001-A; however, no difference was observed in the amount of *P. aeruginosa*, clinical symptoms, or lung function [68].

Conclusions

Studies in previous years have reported that the lungs are a completely sterile organ. Advances in sequencing technology and the development of new tools have enabled us to accurately identify microbial populations in the airways, especially the lungs. Autoimmune and even infectious diseases are caused by a lack of regulation and disruption of immune responses. Evidence suggests that changes in the microbiota of the lungs or intestines can lead to chronic disease or even exacerbate the disease through changes in the microbiota or susceptibility to new infections. Intrinsic immunodeficiency resulting from microbial incompatibility may exacerbate host susceptibility to infection and chronic lung disease. Evidence suggests that specific microbes play a key role in improving immune responses and that microbial incompatibility plays a part in chronic lung diseases such as asthma, COPD, and CF.

Significant advances in recent years have enriched our understanding of pathogen-host interactions as well as the introduction of new treatment strategies, but there remained many challenges for improving therapies. One reality is that the spread of microbial populations that grow in the CF airway, as well as the biofilms they produce, needs further exploration. Expanding knowledge on T cell population dynamics in CF and how to balance infection and regulate T cells in resistant microbial populations, inflammatory damage, and lung function help create new treatment options and define the term "window of opportunity" to target safety processes. Clever strategies are needed to reduce persistent invasive infections and maintain the host's ability to fight chronic infection.

A better understanding of these issues will improve therapies to reduce infection and minimize lung damage and improve the quality of life in CF patients. In the future, therapies may be focused on creating a healthy population of microbiota and increasing their metabolic capacity, as well as altering the lung microbiota to be introduced as a new treatment for chronic lung disease. As a result, the near future reflects both needs and opportunities. Microbial and immune system pathways are extremely important in both health and disease conditions and lead to the design of high-performance therapeutic strategies.

Ethical Considerations

Compliance with ethical guidelines

There is no ethical principle to be considered in doing this research.

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Authors' contributions

Both authors equally contributed in preparing this article.

Conflicts of interest

The authors declared no conflict of interest.

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