



Role of the transforming growth factor (TGF)- β 1 and TGF- β 1 signaling pathway on the pathophysiology of respiratory pneumococcal infections

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Streptococcus pneumoniae, pneumococcus, is the most common cause of community-acquired pneumonia (CAP). CAP is an important infectious disease with high morbidity and mortality, and it is still one of the leading causes of death worldwide. Many genetic factors of the host and various environmental factors surrounding it have been studied as important determinants of the pathophysiology and outcomes of pneumococcal infections. Various cytokines, including transforming growth factor (TGF)- β 1, are involved in different stages of the progression of pneumococcal infection. TGF- β 1 is a cytokine that regulates a wide range of cellular and physiological functions, including immune and inflammatory responses. This cytokine has long been known as an anti-inflammatory cytokine that is critical to preventing the progression of an acute infection to a chronic condition. On the other hand, recent studies have unveiled the diverse roles of TGF- β 1 on different stages of pneumococcal infections other than mitigating inflammation. This review summarizes the recent findings of the role of TGF- β 1 on the pathophysiology of pneumococcal infections, which is fundamental to developing novel therapeutic strategies for such infections in immune-compromised patients.

Keywords: TGF-beta1; Pneumococcus; Inflammation; Fibrosis

INTRODUCTION

Streptococcus pneumoniae, also known as pneumococcus, is a human pathogen normally found in the nasopharynx of healthy individuals. Although pneumococcus is part of the normal flora in the upper respiratory tract, it is a major cause of death and it is responsible for more than a million deaths each year [1]. Children under 5 years of age and adults over the age of 65 are most susceptible populations to pneumococcus and, thus, have the highest morbidity and mortality rates related to this pathogen than any other group [2]. Although

two types of vaccines, pneumococcal conjugate vaccine and pneumococcal polysaccharide vaccine, have been developed, their use is more common in developed regions and their efficacy is still far limited [3-5]. As both vaccines are made with pneumococcal capsular polysaccharides, the immune responses induced by vaccination are only effective against the serotypes included in the vaccine. Moreover, their use has led to an increase in pneumococcal infections with non-vaccine serotypes, a phenomenon called serotype replacement [2,6]. In addition, the vaccine's efficacy is reduced in populations with weaker adaptive immune systems, as is the case for the populations mentioned earlier, as well as in individuals with other immune-compromising conditions [3,6,7]. Therefore, many studies have focused on understanding the early defense mechanisms against pneumococcal infections, and how these mechanisms prevent the dissemination of the pathogen and the development of invasive pneumococcal diseases (IPDs). They also try to develop therapeutic strategies that regulate

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pneumococcal infections, even in cases where the patient has a weaker adaptive immune system.

Transforming growth factor (TGF) is a family of proteins that control a diversity of cell processes, including cell differentiation, cell division, and apoptosis, and have important roles during embryogenesis and development [8]. This family of proteins includes the TGF- β 1, TGF- β 2, TGF- β 3, bone morphogenic proteins (BMPs), activins/inhibins, nodal, growth differentiation factors, and Müllerian inhibiting substances [9]. TGF- β 1 is the most extensively studied protein in the TGF family. Since its first purification at the beginning of the 1980s, studies have reported the role of TGF- β 1 in the regulation of development, as well as in the immune system, tissue regeneration and even the progression of various diseases [10]. The immune regulatory functions of TGF- β 1 have been studied extensively in the adaptive immune system since it was first found to be a master regulator of T cell tolerance; it regulates the proliferation, differentiation, and cellular activities of T cell subsets [11,12]. On the other hand, few studies have examined the role of TGF- β 1 in regulating the innate immune system, particularly against respiratory bacterial infections. The expression of TGF- β 1 has been reported in patients and mice models with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) [13], but the role of TGF- β 1 on the pathophysiology of respiratory bacterial infections is not completely understood. Many studies have been conducted to understand the role TGF- β 1 plays during the different stages of a pneumococcal infection, which is the most common cause of bacterial ALI/ARDS. This review discusses the research into the role of TGF- β 1 and its signaling pathways in the pathophysiology of pneumococcal infections.

Expression and activation of TGF- β 1

TGF- β 1 is expressed initially as a pre-pro-TGF- β 1, which contains an N-terminal signal peptide that is cleaved for the remaining pro-TGF- β 1 to function. Pro-TGF- β 1 is formed by a pro-domain, called the latency-associated protein (LAP), and the biologically active C-terminal polypeptide [14]. Normally, these two proteins will stay linked, forming the small latent complex. Some cells, however, produce TGF- β 1 and deposit it on the extracellular matrix (ECM) as the large latent complex (LLC). The LLC is formed by an interaction of pro-TGF-

β 1 with the latent TGF- β 1 binding protein, which renders TGF- β 1 inactive [14]. Latent TGF- β 1 is then activated by proteases and integrins, such as matrix metalloproteinase (MMPs) and α v β 6 and α v β 8 integrins [14-17]. Furthermore, proteins present on the ECM, e.g. fibrin, fibronectin, and thrombospondin, can also activate or prevent the function of TGF- β 1 by mechanisms not yet completely defined [11,14].

TGF- β 1 signaling pathways

TGF- β 1 transmits signals through the cell surface type I (T β RI) and type II (T β RII) serine/threonine kinase receptors [18]. T β Rs are normally on the cell surface as homodimers. Once TGF- β 1 binds to the homodimers of T β RII, the T β RI homodimers join them to form a hetero-tetrameric receptor complex [9], which is then phosphorylated by T β RII. The activation of T β Rs by TGF- β 1 triggers the Smad-dependent signaling pathway and many other non-canonical signaling pathways, including ERK1/2, p38 mitogen activated protein kinases (MAPKs) and phosphoinositide 3-kinase (PI3K)-Akt signaling (Fig. 1) [19,20].

1. Smad-dependent signaling pathway

Eight Smad proteins are found in humans and are subdivided into receptor-regulated Smads (R-Smads), common mediator Smad (Co-Smad), and inhibitory Smads (I-Smads). Among them, Smad1, Smad2, Smad3, Smad5, and Smad8 are R-Smads; Smad4 is Co-Smad, and Smad 6 and Smad7 are I-Smads [18]. Generally, Smad2 and Smad3 are R-Smads activated by TGF- β 1 [14] once they are phosphorylated by activated T β RI [11]. Phosphorylated R-Smads then interact with Smad4 and translocate into the nucleus where they interact with transcription factors and initiate the transcription of TGF- β 1 target genes [8,9,21]. Ultimately, the genes that are activated depend on the type of cells being stimulated and the type of transcription factors interacting with the Smad proteins.

Activated T β R complexes are internalized through endocytosis either at the clathrin-coated pits or caveolin-positive lipid rafts [22]. The receptors at the clathrin-coated pits are guided to early endosomes filled with a Smad anchor for receptor activation protein, which recycles and returns T β Rs to the

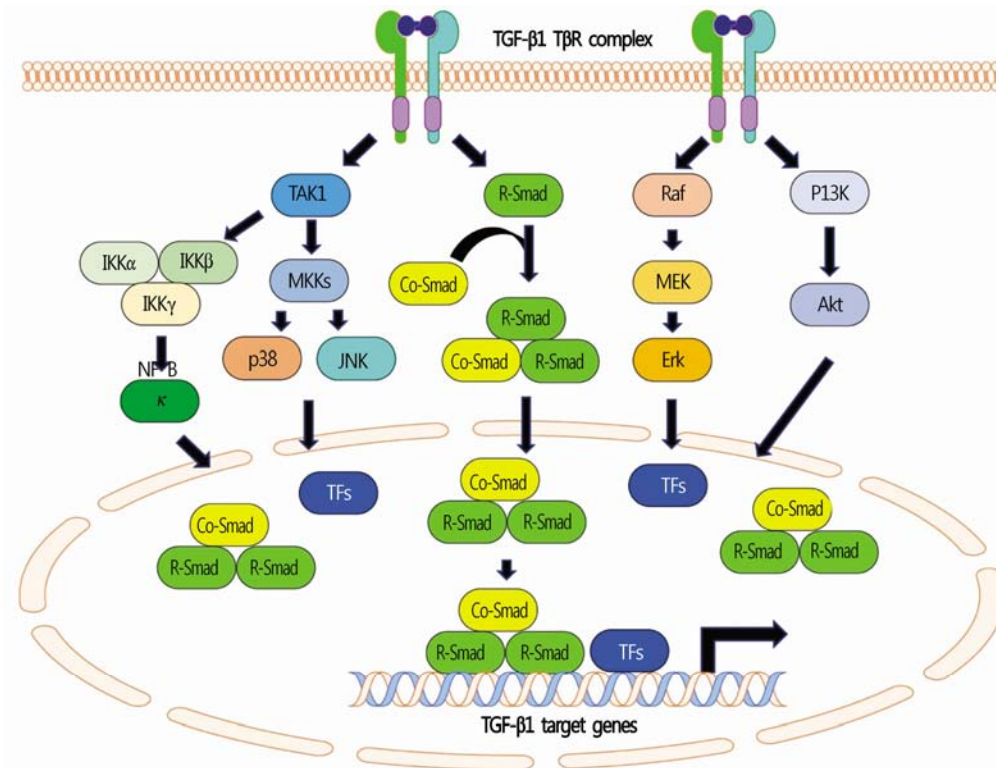


Fig. 1. Smad-dependent and Smad-independent signaling pathways activated by TGF- β 1. TGF- β 1 binds and activates the cell surface receptors, T β Rs, and activated T β Rs transmit signals through Smad-dependent and Smad-independent signaling pathways. In Smad-dependent pathway, receptor-regulated Smads (R-Smads) are phosphorylated by activated T β RI, which then form heterotrimeric complex with common mediator Smad (Co-Smad) and translocate into the nucleus. In Smad-independent pathways, multiple kinases including p38, Erk, and JNK mitogen activated protein kinases (MAPKs), inhibitor of nuclear factor- κ B kinase β , and Akt are activated *via* TGF- β -activated kinase 1, Raf, and phosphoinositide 3-kinase. All these individual pathways regulate the expression of TGF- β 1 target genes by regulating their own transcription factors, but they also directly phosphorylate Smad proteins, converging both Smad-dependent and Smad-independent pathways. TAK1, TGF- β -activated kinase 1; IKK, inhibitor of nuclear factor- κ B kinase; NF- κ B, nuclear factor- κ B; MKK, MAPK kinase; PI3K, phosphoinositide 3-kinase; TFs, transcription factors.

cell surface. On the other hand, caveolin-positive lipid rafts form vesicles containing I-Smads and Smad-ubiquitination-regulatory factors (Smurfs) to mark T β Rs for degradation [22].

2. Smad-independent signaling pathway

In addition to the Smad proteins, phosphorylated T β Rs activate MAPK kinase kinases (MAP3Ks) including TGF- β -activated kinase 1 (TAK1) and Raf, which play critical roles in the TGF- β 1-induced Smad-independent signaling pathways [19]. Activated TAK1 activates MAPK kinase and the inhibitor of nuclear factor (NF)- κ B β , which results in subsequent activation of MAPKs, such as JNK and p38 MAPKs, and NF- κ B. The activation of Raf by TGF- β 1 stimulates MEK1/2, which then activates Erk1/2 MAPK. In addition to the activation of

the MAPKs and NF- κ B signaling pathways, TGF- β 1 also stimulates PI3K, which then activates Akt [20]. Although all these individual pathways regulate the expression of the TGF- β 1 target genes by regulating their own target transcription factors, they can also phosphorylate Smad proteins directly, converging both Smad-dependent and Smad-independent pathways [9,23].

The signaling pathways activated by TGF- β 1 also crosstalk with many others, for example, with the innate immune signaling pathways induced by Interleukin-1 receptor (IL-1R) and Toll-like receptors (TLRs). IL-1R and TLRs activate the pathways mediated by TNF receptor-associated factor 6 (TRAF6) and TAK1, which are fundamental for the TGF- β 1-induced activation of the JNK/p38 MAPK pathways [19,20].

Regulation of the immune cells by the TGF-β1 and TGF-β signaling pathways

Invading microbes first encounter tissue resident macrophages and epithelial cells. The sensing of microbes by these cells triggers the innate immune response by producing a range of cytokines and acute phase proteins, which then recruit circulating monocytes and neutrophils. At the same time, epithelial dendritic cells phagocytose microbes and activate the adaptive immune T and B cells [24,25]. TGF-β1 acts as a positive or negative regulator of the immune/inflammatory responses by regulating the activation, proliferation, and differentiation of these cells (Fig. 2).

1. Epithelial cells

Respiratory epithelial cells are threatened continuously by airborne microbes, and therefore well equipped with a strong innate immune system [26]. Pathogenic factors are sensed by epithelial cells via the cell surface expressing pattern recognition receptors (PRRs) including TLRs, and intracellular PRRs, which detect invading intracellular microbes and their components. The recognition of microbial factors stimulates the cells to produce other PRRs, acute phase proteins and cytokines, to recognize the microbes more efficiently, inhibit bacterial growth, recruit inflammatory cells to the site of infec-

tion, and to enhance the phagocytic activity [24]. Alveolar epithelium expresses TGF-β1, which is upregulated further upon an infection with respiratory bacterial pathogens, including *Mycobacterium tuberculosis* (*M. tuberculosis*) [27-29], *Pseudomonas aeruginosa* [30-33], nontypeable *Haemophilus influenzae* (NTHi) [34-36], and pneumococcus [35,37].

At the early stages of infection, TGF-β1 positively regulates the epithelial inflammatory responses in the airway epithelium by enhancing the transcriptional activity of NF-κB and enhancing TLR2 expression [38-40]. TGF-β1 synergistically enhances the NTHi- and pro-inflammatory cytokine tumor necrosis factor (TNF)-α-induced inflammation *via* the acetylation of p65, which results in enhanced NTHi- and TNF-α-induced-NF-κB activation and subsequent inflammation [38,39]. The epithelial cell expression of TLR2 is upregulated following NTHi infection, but TLR2 expression is under tight control by p38 MAPK to avoid detrimental inflammatory responses [40]. On the other hand, activation of the TGF-β1 signaling pathway by a NTHi infection upregulates the MAPK phosphatase (MKP)-1 expression, which then inhibits the p38-mediated inhibition of TLR2 expression, thereby promoting inflammation. In addition, TGF-β1 increases the epithelial permeability by downregulating epithelial tight junction integrity, and promotes the migration of inflammatory cells from the blood to the airway and the leakage of anti-microbial molecules from the circulation [41-43]. These findings in the

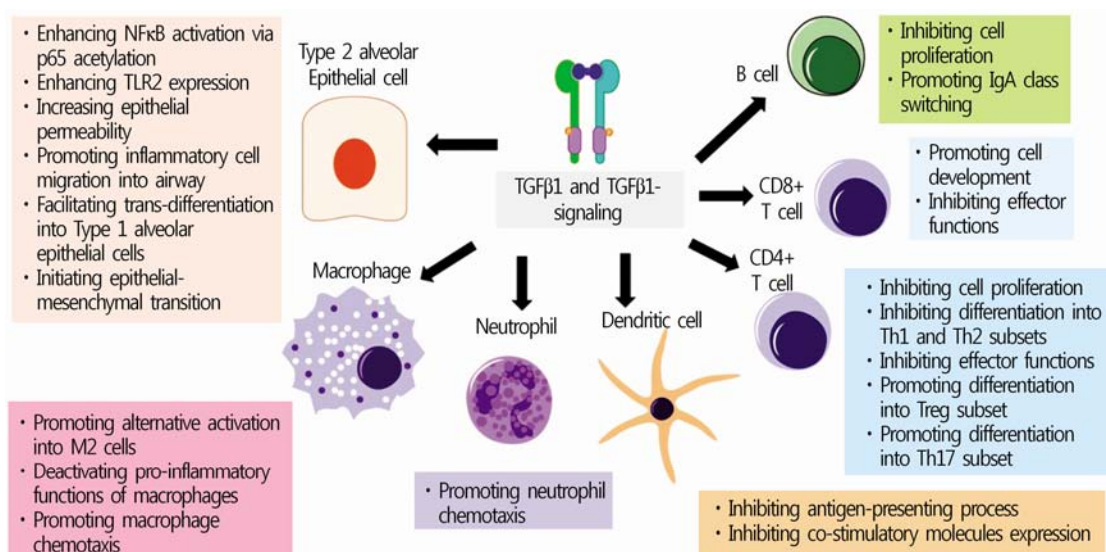


Fig. 2. Immune modulatory functions of TGF-β1 on cells in the airway. The role of TGF-β1 on airway epithelial cells, macrophages, neutrophils, dendritic cells, CD4 and CD8 expressing T cells, and B cells are summarized.

airway epithelium suggest that TGF- β 1 acts as a positive regulator for airway inflammation during respiratory bacterial infections, in contrast to its well-known inflammation mitigating effect.

Following an acute inflammation process, type II alveolar epithelial cells differentiate into type I alveolar epithelial cells and fibroblasts to regenerate the epithelial structures damaged due to acute and severe infections [44-46]. The TGF- β 1 and TGF- β 1 signaling pathways assist in the tissue regeneration processes by stimulating the trans-differentiation of type II alveolar epithelial cells to type I alveolar epithelial cells [45]. TGF- β 1 also facilitates the tissue regeneration processes by inducing an epithelial-mesenchymal transition (EMT). Transcription factors induced by TGF- β 1, such as Snail and Slug, inhibit the expression of tight junction proteins and E-cadherin, thereby stimulating the trans-differentiation of type II alveolar epithelial cells to mesenchymal cells expressing N-cadherin and producing ECM [47,48]. The regulation of epithelial cell differentiation by TGF- β 1 is necessary to regenerate the damaged epithelium, but these processes often result in tissue destruction and pulmonary fibrosis [49,50].

2. Phagocytes

Phagocytes, such as macrophages and neutrophils, are the most effective cells in removing microbes. Alveolar macrophages are the most abundant phagocytes in the normal alveolar space without an active infection. In the resting condition, the phagocytic activity of resident macrophages is suppressed by TGF- β 1, which is believed to be a major reason for their relatively long lifespan in tissues [51]. Once they encounter microbes, the expression of T β Rs is downregulated, leaving them less responsive to TGF- β 1. Activated alveolar macrophages phagocyte and kill invading microbes and secrete pro-inflammatory cytokines to recruit circulating monocytes/macrophages and neutrophils [16]. Following extravasation, macrophages undergo phenotypic polarization processes and develop into distinctive subsets of macrophages. The specific phenotypic polarization of macrophages is determined by the microenvironmental signals, such as the type of activator and the type of cytokines activating macrophage polarization. TLR ligands along with interferon (IFN)- γ lead to the classical activation of macrophages, which results in macrophages expressing pro-inflammatory cytokines, and ha-

ving anti-microbial activities (the so called M1 macrophages). If macrophages are polarized under IL-4 and IL-13-rich conditions, they are polarized into cells expressing IL-10 and TGF- β 1. This polarization process is called alternative activation, and results in heterogeneous and functionally distinct macrophage subsets, known as M2 macrophages [52].

Although the removal of invading microbes by phagocytes is essential to control an infection, the surrounding tissues are damaged by the byproducts of phagocytosis including toxic reactive oxygen species, mammalian toxic proteases, and nucleases [25]. Alternatively activated M2 cells clean up dead neutrophils and damaged epithelial cells during inflammation, and once the clearance of apoptotic cells, called efferocytosis, begins, it signals the deactivation of the inflammatory process [51]. Although the precise mechanism used for TGF- β 1 deactivation of the inflammatory response is unclear, TGF- β 1 was found to promote the ubiquitination and degradation of MyD88, a master adaptor molecule for TLRs activation except for TLR3, as well as downregulate the expression of the major histocompatibility complex class II [52]. In addition, neutrophil chemotaxis depends on the TGF- β 1 and TGF- β 1 signaling pathway because the neutrophils in *Smad3*^{-/-} mice had an impaired chemotactic response and the production of IL-8, a chemokine necessary for the migration of neutrophils, was blocked [12]. Therefore, at early stages of bacterial infections in the lung, TGF- β 1 may facilitate the acute inflammatory responses by promoting the extravasation of phagocytes, and promoting the resolution of inflammation by deactivating the phagocytes and promoting their M2 polarization.

3. Dendritic cells

DCs are present in non-lymphoid tissues, such as the lungs, as immature DCs [53]. In non-lymphoid tissues, the DCs phagocyte antigens, such as invading microbes, and migrate to the secondary lymphoid organs to present captured antigens to T cells and activate them [53]. DCs produce considerable amounts of TGF- β 1 [54,55], but the effects of TGF- β 1 on the function and activation of DCs are not completely understood. On the other hand, a series of experimental data suggest that TGF- β 1 and the TGF- β 1 signaling pathways function mainly as negative regulators of the self-immune responses because TGF- β 1 inhibits the antigen-presenting process and the expression of co-stimulatory molecules on DCs

[56,57]. Furthermore, the activation of DCs by TGF- β 1 is dependent largely on the cell surface expression of integrin $\alpha\beta$ 8, and the loss of $\alpha\beta$ 8 on DCs results in dysregulated immune responses, including a decrease in regulatory T (Treg) cells [57]. This suggests that TGF- β 1 has important roles on DCs activation and subsequent regulation of T cell homeostasis. In contrast, Laouar et al. showed that the DC-specific inhibition of TGF- β 1 signaling had no effects on the DC function [58]. The role of TGF- β 1 and the TGF- β 1 signaling pathways on the cellular and immunological functions of DCs needs to be investigated further, particularly in response to specific bacterial pathogens.

4. T and B cells

TGF- β 1 is fundamental to the regulation of T and B cells [11,12]. Helper T (Th) cells expressing CD4 are differentiated into different Th cell subsets including Th1, Th2, Th17, and Treg depending on the complex interaction between the DC-presenting antigens and cytokines participating in the activation process. Th1 cells expressing IFN- γ enhance the phagocytic activities of macrophages, and Th2 cells expressing IL-4 and 5 stimulate B cell activation to produce antigen-specific antibodies. The Th17 cells express IL-17 and are subdivided further into regulatory Th17 cells, expressing IL-9 and IL-10, and pathogenic Th17 cells, expressing IFN- γ and granulocyte-macrophage colony-stimulating factor [59]. Treg cells inhibit the effector functions of the T cell subsets, DCs, and macrophages via IL-10, IL-35, and TGF- β 1 [60,61]. TGF- β 1 blocks the transcription factors T-Bet and GATA-3 in CD4 expressing Th cells, preventing the differentiation of Th cells into Th1 and Th2, thereby stimulating the generation of Treg cells [62]. Furthermore, TGF- β 1 stimulates the expression of Foxp3, a transcription factor involved in generation of Treg cells and in the inhibition of pro-inflammatory cytokine production and enhancement of anti-inflammatory cytokines like IL-10 and TGF- β 1 [63]. TGF- β 1 is essential for preventing Th17 cell differentiation into the pathogenic Th17 subset and to keep it with a regulatory Th17 phenotype [59]. In addition, in thymocytes, TGF- β 1 promotes IL-7R α expression, and the signaling through this receptor, together with one from the T cell receptor, is required for the development of CD8⁺ T cells [64,65]. On the other hand, TGF- β 1 inhibits the differentiation of CD8⁺ T cells into cytotoxic T lymphocytes [65].

TGF- β 1 inhibits the growth of B cells and activates their apoptotic pathways through the downregulation of NF- κ B/Rel [66]. Although TGF- β 1 was found to inhibit immunoglobulin synthesis, it also enhances the presentation of antigens to B cells and induces a class switch of immunoglobulin M (IgM) to IgA [62] for the immune defense of mucosal surfaces. Experiments that specifically block B cell-specific TGF- β 1 showed a decrease in IgA production, which could be emulated by blocking Smad2, while a Smad3 deficiency did not affect the levels of IgA [12].

Overall, TGF- β 1 functions mainly as a repressor to restrict the pro-inflammatory functions of T cells by limiting Th1 and Th2 cell differentiation and by promoting Treg and regulatory Th17 cell differentiation. On the other hand, TGF- β 1 may play a critical role in the mucosal immunology, which is a key defense mechanism in respiratory bacterial infections, by enhancing IgA secretion.

Regulation of pneumococcal infections by the TGF- β 1 and TGF- β 1 signaling pathway

1. Pathophysiology of respiratory pneumococcal infections

The first point of entry for pneumococcus is through the upper respiratory system, which is colonized by pneumococcus prior to infection. Attachment to the surface of epithelial cells is the first step in establishing an infection. Multiple pneumococcal factors, including pneumococcal surface adhesion A, choline binding protein A (CbpA), and pneumococcal phosphorylcholine (ChoP), and the host receptors, such as sialic acid, polymeric immunoglobulin receptor (pIgR), and platelet-activating factor receptor (PAF-R), are involved in this process [67]. The prolonged colonization and delayed clearance of pneumococcus results in overgrowth of the bacteria on the epithelial surface [68]. Therefore, the timely removal of epithelial pneumococcus is also the first step in inhibiting pneumococcal infections. Epithelial cells respond to pneumococci by producing various antimicrobial molecules to directly kill or opsonize them. The opsonized pneumococcus is phagocytosed by macrophages, neutrophils, and DCs [1]. Epithelial cells also produce pro-inflammatory cytokines and chemokines to recruit and activate phagocytes from the blood [69].

Therefore, after successful colonization, pneumococcus must invade the epithelial layers to avoid the mucosal defense mechanisms. Among the many pneumococcal pathogenic factors, the cytoplasmic toxin pneumolysin has been considered a major virulence factor facilitating the dissemination of pneumococcus inside the body [70]. Pneumolysin binds to cholesterol on epithelial cells, and oligomerization of the pneumolysin-cholesterol complexes form membrane pores large enough to lysis the cell [71]. The loss of epithelial cell barrier integrity and enhanced epithelial permeability lead to the trans-epithelial migration of pneumococcus to the bloodstream. Plasmin, the activated form of plasminogen, binds to pneumococcus, which leads to the cleavage of tight junctions and the subsequent migration of pneumococcus through the epithelial layer [72]. Pneumococcus can also be internalized into epithelial cells via several different mechanisms. Pneumococcal binding to the ECM component, vitronectin, facilitates the intra-epithelial and endothelial translocation of pneumococcus [73]. Pneumococcal surface protein C and CbpA bind to the pIgR on the epithelial cell surface and facilitate the internalization of pneumococcus [74]. The epithelial internalization of pneumococcus is also achieved through binding to the PAF-R and laminin receptor via ChoP and CbpA, respectively [75].

Once a pneumococcus enters the circulation, circulating anti-bacterial proteins, including C-reactive protein, long pentraxin 3, complements, and antibodies, bind to pneumococcus. Opsonization with these proteins enhances phagocytosis by macrophages and neutrophils. As they enter the circulation, pneumococcus can also cross the endothelium and enter other tissues, e.g., the subarachnoid space through the blood-brain barrier [76]. Once they reach the central nervous system, the damages caused by pneumococcus and the inflammatory response are detrimental regardless of the extent of the damage. The bacteria can be removed effectively if treatment is initiated and the pneumococcal strain causing the infection is susceptible to the antibiotics introduced. On the other hand, sterile inflammation and even septic shock due to the released pneumococcal pathogenic factors from dead cells are often observed.

2. Regulation of pneumococcal infections by the TGF- β 1 and TGF- β 1 signaling pathways

The enhanced expression of TGF- β 1 has been found in

the bronchoalveolar lavage fluid and blood from patients with a pneumococcal infection and animal models of pneumococcal infections [77,78]. Pneumococcus stimulates the production of TGF- β 1 from the nasopharyngeal epithelial cells and fibroblasts, which in turn activates T cell differentiation into Treg cells and played critical roles for the prolonged colonization and delayed clearance of pneumococcus from the nasopharynx [78]. In addition, the prolonged colonization of pneumococcus in the nasopharynx facilitates the alternative activation of macrophages into M2 macrophages, limiting the pro-inflammatory responses in the nasopharynx and allowing the persistence of pneumococcal carriage [78]. Therefore, the pneumococci appear to employ the host anti-inflammatory and anti-immune responses by stimulating the production of TGF- β 1, and successfully colonizing the nasopharynx without causing inflammation. On the other hand, clinical studies with pneumococcal infection-prone children also showed the important role of TGF- β 1 on limiting pneumococcal infections in these patients [79,80]. Low nasopharyngeal colonization by pneumococcus was associated with enhanced Th17 cell immunity [79], whereas repeated infections with pneumococcus was associated with a significant defect on the differentiation of blood mononuclear cells to Th17 cells following pneumococcal infections [80]. Furthermore, the addition of exogenous cytokines promoting Th17 polarization including TGF- β 1 could rescue the cells to differentiate into Th17 cells, which suggests that TGF- β 1 inhibits pneumococcal colonization in the nasopharynx by activating Th17 cells [80]. Such controversial findings on the role of TGF- β 1 in pneumococcal colonization need to be investigated further experimentally.

Following the successful colonization of the nasopharynx, pneumococcus must translocate into the body to establish an infection. Pneumococcus activates the p38 MAPK and TGF- β 1 signaling pathways to transmigrate through the epithelial barrier [42]. Pneumococcal colonization was reported to enhance and activate transcription factor Snail-1, which is under the control of TGF- β 1. Snail-1 inhibits E-cadherin and ultimately causes the loss of the tight junctions and opens the epithelial barrier, leaving the tissue vulnerable to bacteria [43]. During a pneumococcal infection, the loss of tight junctions was also caused by the Snail-1 dependent downregulation of claudins, which are the key components of the tight junctions [34]. Although pneumococcus induced the activation of the TGF- β 1 signaling pathway, and transepithelial migration of

pneumococcus was blocked by the T β R1 inhibitor, SB431542 [42], it is still unclear whether the production of TGF- β 1 is involved or T β R1-dependent activation of TGF- β 1 signaling pathway is activated directly by pneumococcus. Another important respiratory bacterial pathogen, NTHi, was found to activate T β R1-dependent TGF- β 1 signaling pathway directly *via* the expression of a TGF- β 1-like molecule [40]. In addition, the expression of PAI-1, an acute phase protein regulated mainly via the TGF- β 1 signaling pathway, is also induced directly by the pneumococcal toxin pneumolysin [81]. Therefore, it would be interesting to know if pneumococcus also activates the TGF- β 1 signaling pathway directly by expressing a TGF- β 1-like molecule, as NTHi does, or if T β Rs are activated by pneumococcus via another mechanism that is not completely understood.

Following acute inflammation and injury during a pneumococcal infection, the removal of damaged cells and edematous solution from the alveolar space is important to minimize tissue damage [82]. Efferocytosis against apoptotic and necrotic cells is conducted mainly by alternatively activated M2 macrophages, and TGF- β 1 promotes efferocytosis by stimulating alternative M2 activation [83]. TGF- β 1 also promotes tissue regeneration by stimulating the trans-differentiation of type 2 epithelial cells to type 1 epithelial cells and sealing the denuded epithelial layer by stimulating the production of ECM, like collagen, and by stimulating the EMT process to supply fibroblasts to the surrounding injured tissues [45-48]. Although this is important for tissue repair, the uncontrolled accumulation of collagen and the EMT process can lead to further complications, such as lung fibrosis [49,50]. Therefore, the expression of TGF- β 1 and activation of the TGF- β 1 signaling pathways are tightly regulated. For example, GSK3 β -STUB1 maintains a low epithelial cell response to TGF- β 1 by ubiquitinating and degrading Smad3 [49,84]. Following a pneumococcal infection, however, pneumococcus activates Akt, which in turn deactivates GSK3 β and inhibits the GSK3 β -STUB1-dependent degradation of Smad3 [49]. This process enhances the epithelial responsiveness to TGF- β 1 and promotes the production of the TGF- β 1-response genes, such as collagen and PAI-1. The protein deubiquitinase CYLD suppresses this process by deubiquitinating and inhibiting Akt. Nevertheless, if the inhibitory effect of CYLD is lost, lungs infected with and damaged by pneumococcus undergo fibrotic changes due to the enhanced responsiveness to TGF- β 1 [49]. As a result,

fibrotic scar tissue is often found in the lungs of patients following severe pneumococcal infections, which indicates a failure of the tight regulation of the tissue regeneration process in these patients [49].

Excess extracellular water inhibits the normal lung functions, such as gas exchange, and inhibits the lung defense mechanisms against invading microbes. Pulmonary edema forms when the increase in lung vascular permeability cannot be opposed by the active clearance of fluid by the epithelium [85]. Clearance of this fluid is achieved through osmosis *via* a sodium gradient produced by sodium channels, such as the epithelial sodium channels (ENaCs) [86]. The enhanced expression of TGF- β 1 was observed in the bronchoalveolar lavage fluids of ALI/ARDS patients and mice, and TGF- β 1 signaling causes the internalization of ENaCs by inducing endocytosis of its β subunit, which stabilizes the $\alpha\beta\gamma$ ENaC complex on the cell membrane [86]. TGF- β 1 also inhibited the mRNA expression of the α subunit of ENaC (α ENaC) [87], thereby decreasing the movement of sodium across the alveolar membrane, disrupting the sodium gradient and prolonging alveolar edema [88]. Prolonged alveolar edema eventually induces hypoxic epithelial cell injury and death.

CONCLUSION

TGF- β 1 is a highly conserved structure across species that diverged from one another millions of years ago, which highlights the great importance of these protein. Moreover, it is strongly involved in multiple signaling pathways and its effects can be just as varied. When examining the effects of TGF- β 1 on respiratory bacterial infections, a pattern clearly appears; TGF- β 1 plays a role in the initial inflammatory response and its resolution. On the other hand, when its pro-inflammatory effects persist during the resolution phase, TGF- β 1 stimulates the EMT, leading to pulmonary fibrosis. Pneumococcus appears to actively employ the TGF- β 1 and TGF- β 1 signaling pathways to successfully colonize the nasopharynx by suppressing the anti-bacterial mucosal immune/inflammatory responses. When the mucosal immune responses are activated, the pneumococci activate the TGF- β 1 signaling pathways to disrupt the epithelial barrier integrity and facilitate its dissemination into the circulation. Moreover, TGF- β 1 inhibits the alveolar fluid clearance, which results in hypoxic epithelial injury and impaired mucosal anti-microbial defense responses.

During the recovery and tissue regeneration processes, pneumococci enhances the cellular responsiveness to TGF- β 1 and activation of the TGF- β 1 signaling pathways, which results in aberrant tissue remodeling and the development of fibrotic scarring in lung tissues. Because TGF- β 1 and TGF- β 1 signaling pathways have varying roles at the different stages of pneumococcal infections, it is unclear if inhibition or activation of TGF- β 1 and TGF- β 1 signaling pathway would be beneficial to the host. Nevertheless, at least in the mice model of pneumococcal infections, specific inhibitions of TGF- β 1 or T β Rs effectively prevented the transepithelial dissemination of pneumococcus and the development of IPDs at the early stages of infection and prevented the development of lung fibrosis at the later stages of infection. Nevertheless, this gives researchers the possibility of finding a treatment for pneumococcal infections by targeting the TGF- β 1 and TGF- β 1 signaling pathways.

After decades of research, there are still many details regarding the precise mechanisms of TGF- β 1 and the exact reason why one function or other is expressed at a particular moment. Further studies will be needed to gain a complete understanding of this cytokine and the ways in which its signaling can be influenced.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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