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Respiratory tract barrier dysfunction in viral-bacterial co-infection cases☆

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ABSTRACT

A preceding viral infection of the respiratory tract predisposes the host to secondary bacterial pneumonia, known as a major cause of morbidity and mortality. However, the underlying mechanism of the viral-bacterial synergy that leads to disease progression has remained elusive, thus hampering the production of effective prophylactic and therapeutic intervention options. In addition to viral-induced airway epithelial damage, which allows dissemination of bacteria to the lower respiratory tract and increases their invasiveness, dysfunction of immune defense following a viral infection has been implicated as a factor for enhanced susceptibility to secondary bacterial infections. Given the proximity of the oral cavity to the respiratory tract, where viruses enter and replicate, it is also well-established that oral health status can significantly influence the initiation, progression, and pathology of respiratory viral infections. This review was conducted to focus on the dysfunction of the respiratory barrier, which plays a crucial role in providing physical and secretory barriers as well as immune defense in the context of viral-bacterial synergy. Greater understanding of barrier response to viral-bacterial co-infections, will ultimately lead to development of effective, broad-spectrum therapeutic approaches for prevention of enhanced susceptibility to these pathogens.

1. Introduction

Respiratory tract infections are extremely frequent in both children and adults, causing an economic burden on healthcare systems, as well as increased morbidity and mortality [1]. As for the microbial etiology, bacteria, viruses, fungi, and parasites are known causative agents. The respiratory tract is a complex organ divided into the upper and lower respiratory tracts, with the former comprised of the nasal cavity, pharynx and larynx, and the latter the conducting airways (trachea, bronchi, and bronchioles) and respiratory zone (respiratory bronchioles and alveoli). Each component has a specific function and regional differences in cellular composition reflect their characteristics. The average human inhales more than 10,000 liters of air each day along with any bacteria, virus, and fungal particles present in aerosols in the environment. Additionally, the human respiratory tract is an important reservoir of bacteria, viruses, and fungi, and known to harbor diverse communities of commensal, opportunistic, and pathogenic microorganisms. Because of its anatomical continuity with the respiratory tract, the oral cavity is particularly considered a potential reservoir for respiratory tract pathogens, and poor oral hygiene is associated with an increased risk of respiratory tract infections in the elderly [2,3]. Accordingly, a variety of physical and cellular barriers are present on the mucosal surface of the respiratory tract for interruption of a microbial invasion [4]. Similar to other mucosal surfaces, the airway epithelium is at an interface with the external environment, thus it possesses a variety of factors for microbial infection prevention.

A preceding or concurrent viral respiratory tract infection can predispose an individual to secondary bacterial co-infection throughout the airway, resulting in increased disease severity and also possible sequela such as pneumonia. Indeed, respiratory viral-bacterial co-infection is associated with worse mortality and morbidity as compared to a viral or bacterial infection alone in elderly as well as chronically ill individuals. The most devastating example of a lethal viral-bacterial synergism is possibly the 1918 pandemic caused by the H1N1 influenza A virus (IAV), known as the ‘Spanish flu’. Estimates from clinical case and autopsy series studies suggest that more than 95% of all related severe illnesses and deaths were complicated by bacterial pathogens, most commonly *Streptococcus pneumoniae* [5]. Even since establishment of the antibiotic era, over one half of patients in the subsequent 1957 H2N2 and 1968 H3N2 pandemics with a severe infection had bacterial complications.

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S. pneumoniae was the predominant bacterial pathogen associated with both the 1918 and 1968 pandemics, whereas *Staphylococcus aureus* accounted for 44% of associated deaths in the 1957 occurrence [6,7]. During the recent 2009 H1N1 pandemic, bacterial pneumonia was found to be a complication in one-quarter to one-half of severe and fatal cases. Despite development of effective vaccines and potent antibacterial agents, it is clear that bacterial co-infection is associated with worse outcomes, with regional variations [8]. Of note, in addition to *S. pneumoniae*, *Porphyromonas gingivalis*, the most important pathogen in periodontitis, was detected in an autopsy lung sample from a patient who died of viral pneumonia with A/H1N1/2009 [9]. As periodontal disease progresses, pathogenic bacteria in the oral cavity and oropharynx continually increase, potentially leading to various types of pneumonia and respiratory infections as they reach the lower respiratory tract and lungs. These findings suggest the importance of maintaining vigilance against infections originating from oral microbes during respiratory virus infections and highlights risk factors such as increased inhalation and poor oral hygiene that are associated with the occurrence of respiratory infections. While it is known that bacterial pneumonia is a major complication of influenza infections, bacterial co- and secondary infections are not limited to influenza viruses, as numerous others, including respiratory syncytial (RS), parainfluenza, rhino, adeno, and human corona viruses, are also associated with secondary bacterial pneumonia [10]. Oral commensal bacteria have been found in the bronchoalveolar lavage fluid of COVID-19 patients [11]. Nevertheless, the results of bacterial co- and secondary infections can be quite different depending on the nature of the primary viral infection.

Complex mechanisms are involved in the pathogenesis of bacterial pneumonia following a viral infection. However, it is generally understood that a preceding viral infection can induce epithelial damage, enhance bacterial colonization in the upper and lower respiratory tract, and cause immune response dysfunction, leading to increased susceptibility to a secondary bacterial infection. Nasopharyngeal bacterial carriage, a largely asymptomatic condition, is also often considered a prerequisite for invasive disease, as it is an important factor for tissue invasion or dissemination into the lower airway tract. Indeed, *S. pneumoniae*, the predominant cause of secondary bacterial pneumoniae, is a common inhabitant of the nasopharynx, with 40–95% of infants and 10–25% of adults colonized at any given time [12]. Oral dysbiosis also increases the presence of virulent and inflammatory microbes, leading to their dissemination into the respiratory tract. This review provides details on how a previous viral infection, particularly IAV infection, predisposes the host to a secondary bacterial infection, with a focus on the two most common pathogens: *S. pneumoniae* and oral bacteria. Additionally, current understanding regarding how modifications and dysfunctions in the respiratory tract barrier following an IAV infection might impair immune response and increase susceptibility to a secondary pneumococcal infection in the host are discussed.

2. Viral infection-induced dysfunction of physical barrier in respiratory tract

2.1. Impairment of mucociliary clearance and salivary barrier

The first barrier that pathogens encounter before reaching the lungs is the mucosa covering the upper airway, which includes the nasal and oral cavities. Epithelium in the airway is composed of ciliated and secretory cells overlaid by a mucus layer that contains various high polymeric mucins [13], antimicrobial peptides, [14] and neutralizing antibodies [15], which provides physical and secretory barriers, and contributes to immune defense. Most inhaled particulates and infectious pathogens are trapped in the mucus layer, and then cleared from the airways by coordinated movement of cilia on epithelial cells in healthy individuals. On the other hand, during respiratory tract infections, including influenza, the large amount of mucus secreted from the airways and lungs can seriously affect the ventilation function of the airways and the exchange of oxygen in the alveoli, leading to hypoxemia and even asphyxia. In that regard, it has been shown that IAV replication in the airway epithelium induces an alteration of mucus production and decreased tracheal mucociliary velocity, resulting in the impairment of mucociliary clearance (Fig. 1) [16]. Given the importance of mucociliary clearance, it is likely that ciliary function impaired by an IAV infection hampers bacterial clearance from the airways, leading to bacterial dissemination into the lower respiratory tract. The overexpression of MUC5AC, a mucin responsible for mucus formation, is a common occurrence in chronic inflammatory respiratory diseases. Notably, the predominant periodontopathic bacteria, *Fusobacterium nucleatum* and *P. gingivalis*, have also been shown to enhance the expression of MUC5AC [17,18]. This could potentially lead to excessive mucus production even in the absence of viral infection. In addition, IAV infection represses secretion of Chitinase-3-like 1 (CHI3L1), an anti-pneumococcal glycoprotein, on the apical surface of bronchial epithelial cells, thereby allowing pneumococcal colonization and rapid growth [19]. Such physiological changes in the respiratory tract are associated with impaired oxygen exchange, airway hyper-reactivity, and dysfunction of normal mechanical clearance of bacteria, which are important problematic factors in patients with chronic obstructive pulmonary disease.

The oral cavity also potentially plays a significant role in viral transmission. Hyposalivation has been identified as a risk factor for acute respiratory infections, indicating that saliva may contribute to the innate immune response during the early stages of infection. Saliva contains various antiviral components, including cathelicidin, lactoferrin, lysozyme, mucins, peroxidase, salivary agglutinin (gp340, DMBT1), sIgA, secretory leukocyte proteinase inhibitor (SLPI), α-defensins, and β-defensins [20]. Salivary gp340 has demonstrated antiviral efficacy against IAV infection [21]. Additionally, the two major antibodies in human saliva are secretory IgA and IgG, both of which are known to play a protective role against influenza viruses. The saliva of healthy elderly individuals is found to contain more Siaα2–3 Gal- and Siaα2–6 Gal-linked receptors than that of children and adults. This suggests that healthy elderly individuals have stronger resistance to IAV, in part due to the higher presence of terminal α2–3/6-linked sialic acid residues in their saliva, which can bind with viral HA and inhibit the activities of IAV. On the other hand, the expression level of terminal α2–3-linked sialic acids in the saliva in elderly individuals with type 2 diabetes mellitus and liver disease was down-regulated [22]. These findings may provide evidence that elderly individuals with chronic diseases, such as diabetes and liver disease, might be more susceptible to influenza infections, especially avian influenza viruses.

2.2. Receptor availability for bacteria

A preceding influenza virus infection can induce not only impairment of mucociliary clearance, but also excess pneumococcal acquisition and carriage in the nasopharynx, which in turn promotes bacterial dissemination to the lower respiratory tract. Virus-induced epithelial damage and exfoliation provide an increased availability of bacterial receptors, resulting in the establishment of bacterial colonization and onset of invasive diseases. The following three mechanisms are considered to be involved in the process (Fig. 2, Table 1).

2.2.1. NA-exposed cryptic receptors

Neuraminidase (NA), an enzyme present on the envelope of influenza viruses, cleaves sialic acid glycoconjugates on airway epithelial cells as well as mucins, allowing viral particles to be released from infected cells and to spread through mucinous secretions. NA-mediated cleavage of sialic acid from the termini of glycochains facilitates both bacterial adherence to cryptic receptors and their proliferation in the upper respiratory tract [23]. Notably, NA proteins are not restricted to viruses. Causative agents of bacterial pneumonia, such as *S. pneumoniae* and oral bacteria, also produce NAs to cleave sialic acids from protective

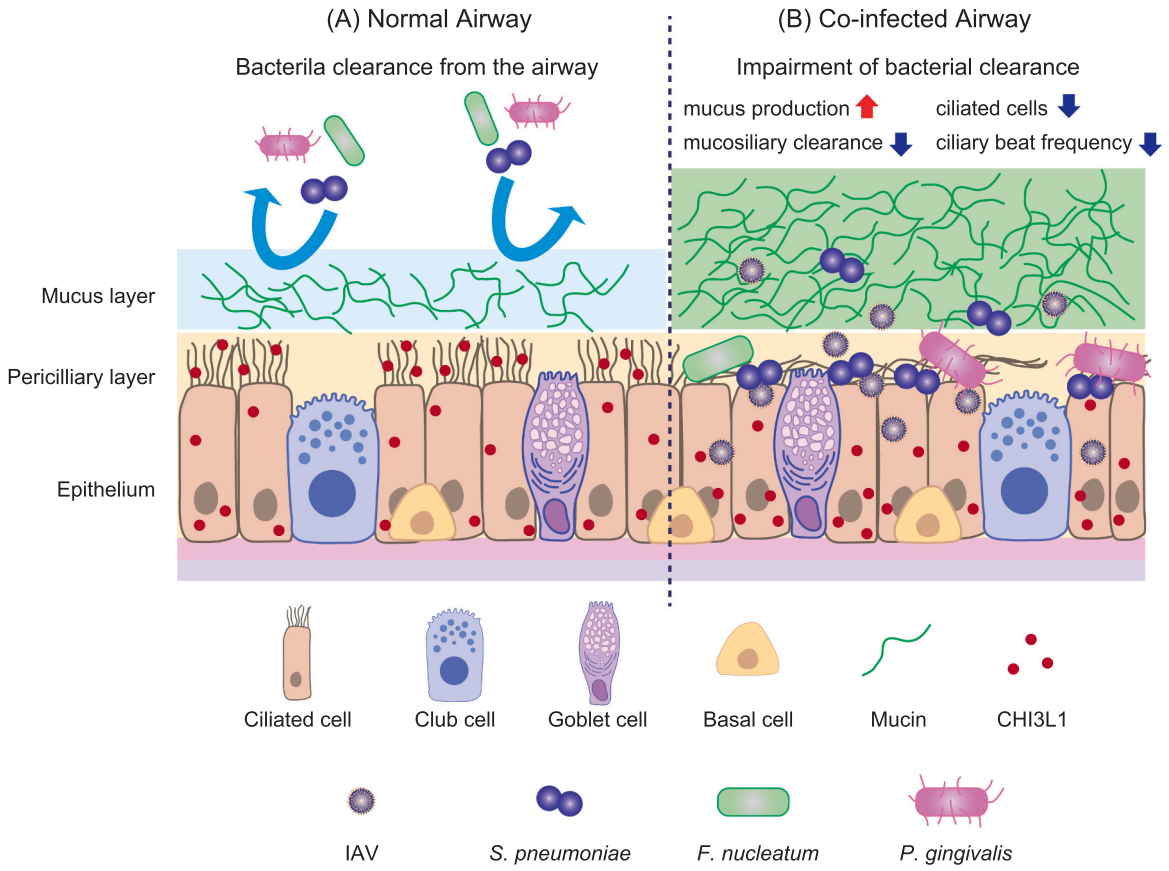

Fig. 1. Impairment of mucociliary clearance in viral-bacterial co-infected airway. (A) Under normal conditions, inhaled particles and infectious agents are trapped in the mucus produced by goblet cells and then cleared from the airway by the coordinated movements of cilia on epithelial cells. (B) During an IAV infection, mucus production is increased to facilitate viral clearance, though conversely, excessive mucus impedes mucociliary clearance. Lower ciliary beat frequency, uncoordinated ciliary movements, and a reduction in the number of ciliated cells are also caused by IAV infection. The major periodontopathic bacteria, *F. nucleatum*, and *P. gingivalis*, can also trigger excessive mucus production, even in the absence of viral infection. It is likely that such reduced ciliary function hampers bacterial clearance. Furthermore, the loss of IAV-induced apical CHI3L1 secretion promotes S. pneumoniae replication during a secondary bacterial infection.

mucins, resulting in efficient bacterial association with receptors on airway epithelial cells and prevention of mucociliary clearance [24]. Although *Streptococcus gordonii*, a pioneer species within the oral cavity, does not encode an NA, it utilizes the NA produced by *S. oralis* to adhere to oral epithelial cells [25]. The assumption is that a prior influenza virus infection in the respiratory tract or inadequate oral hygiene before bacterial invasion creates a conducive environment for accessing the lower respiratory tract. Therefore, the combined effect of viral and bacterial NAs at the site of infection is considered an important step in the synergism of co-infection.

During co-infection, viral proteins displayed on the airway cells have also been reported to facilitate bacterial colonization and pathogenesis. For *S. pneumoniae*, direct binding of the RS virus to the pneumococcal surface through penicillin-binding protein 1a is known to enhance bacterial adherence to epithelial cells and shown to mediate disease with increased severity in a murine model [26]. We also previously reported that *S. pyogenes* binds to IAV particles on epithelial cell surfaces [27]. Furthermore, a recent study showed direct interactions of IAV on the surface of *S. pneumoniae* and *S. aureus* with Gram-positive bacteria, as well as the Gram-negative bacteria *Moraxella catarrhalis* and non-typeable *Haemophilus influenzae*, bacterial colonizers and pathogens in the respiratory tract [28]. These observations support the presence of an additional mechanism related to bacteria-influenza virus synergy at the earliest steps of pathogenesis.

2.2.2. *Surface display of receptors induced by inflammatory response*

Inflammation responses induced by viral infection modify the regulatory state and surface display of multiple proteins on infected cells, thereby facilitating bacterial dissemination to the lower respiratory tract.

Notably, a G protein-coupled receptor, platelet-activating factor receptor (PAFR), has been shown to be exposed on infected epithelial and endothelial cells during IAV infection. Extracellular PAFR binds to phosphorylcholine embedded in the cell walls of numerous respiratory bacterial pathogens such as *S. pneumoniae* and non-typeable *H. influenzae*, then subsequently accelerates lung bacterial burden and bacteremia, increasing mortality risk [29,30]. In addition to a preceding viral infection, culture supernatants from *P. gingivalis* and *Prevotella intermedia*, the major periodontopathic bacterial species, have been shown to enhance PAFR expression in alveolar epithelial cells. Therefore, the aspiration of these periodontopathic bacteria into the lower respiratory tract might constitute a risk factor for severe pneumococcal pneumonia [31,32]. Among the bacterial receptors that appear on cell surfaces during influenza infection, PAFR has gained attention as a possible therapeutic target. For example, *S*-carboxymethylcysteine is a mucolytic agent used for chronic obstructive pulmonary disease shown to inhibit bacterial adherence mediated by phosphorylcholine and PAFR [33]. Similar to influenza, treatment targeting the microbe-host interaction could be a novel strategy for viral-bacterial co-infection. On the other hand, a couple of studies have reported that genetic knockout or pharmacological inhibition of PAFR had no effect on susceptibility of mice to secondary bacterial pneumonia, implicating multifaceted mechanisms and receptors related to the synergism between influenza viruses and bacterial pathogens [34,35].

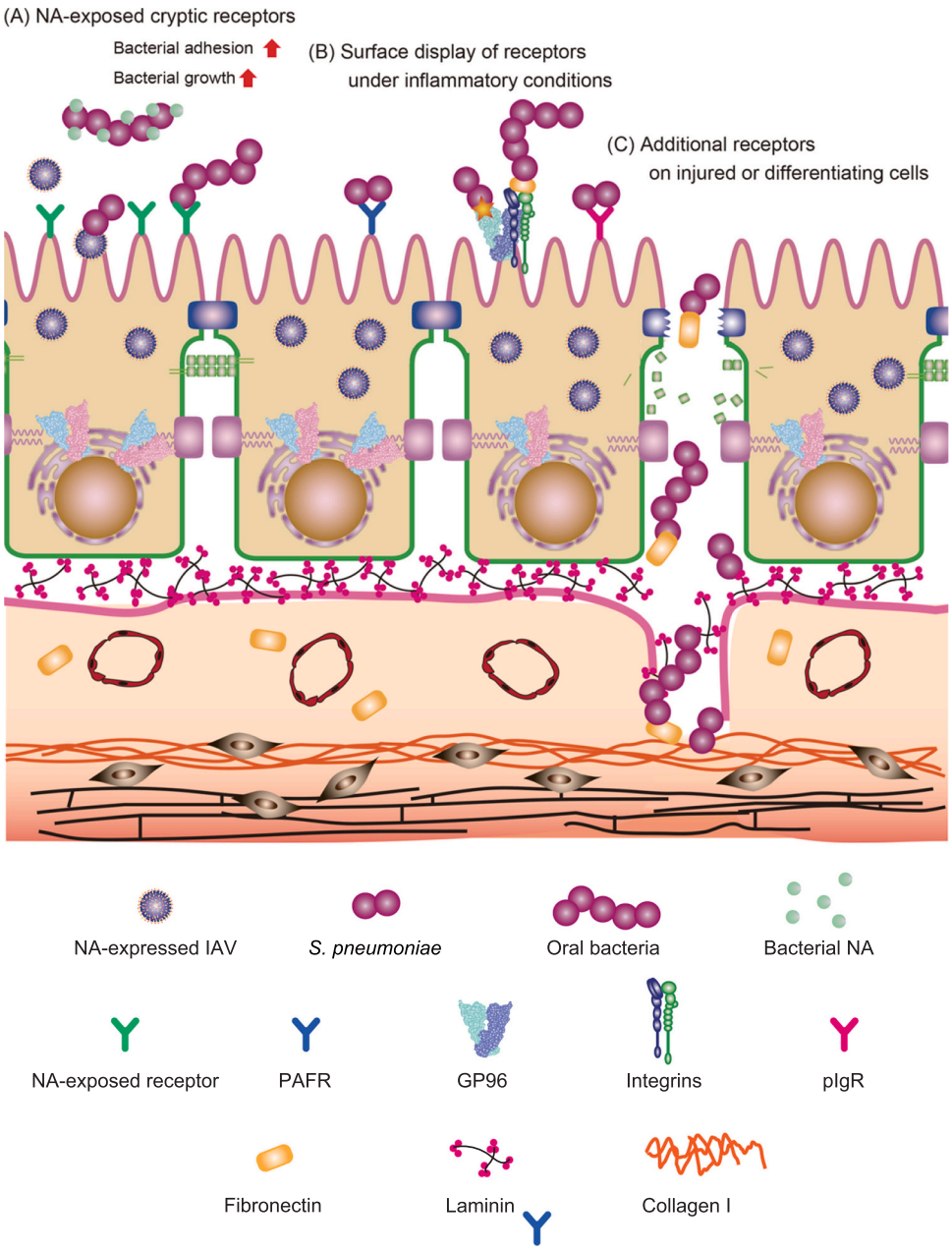

Fig. 2. Receptors availability for bacteria on IAV-infected airway epithelial cells. Bacteria responsible for secondary pneumonia utilize three mechanisms for the establishment of bacterial colonization at virus-infected epithelial cells. (A) First, cryptic receptors are exposed through the enzymatic activity of viral or bacterial NAs, which cleave terminal sialic acids away from cell surface glycoconjugates. (B) Second, IAV infection-induced inflammation up-regulates inactive receptors under inflammatory responses. (C) Third, fibronectin, collagen, and other matrix elements deposited during the regenerative process following viral infection provide attachment sites for bacteria.

During IAV infection, increased pulmonary IFN-γ levels upregulate the polymeric immunoglobulin receptor (pIgR), resulting in pneumococcal adherence to epithelial cells [36,37]. pIgR functions in the epithelial transcytosis of mucosal antibodies and the excretion of antigens and pathogens across mucosal epithelia. The interaction between pIgR and a pneumococcal adhesin, CbpA, has been shown to induce *S. pneumoniae* invasion into nasopharyngeal epithelial cells and the bloodstream through a process termed reverse transcytosis [38]. While the CbpA-pIgR interaction has not been characterized as the pathogenesis of co-infection to our knowledge, it deserves investigation, particularly in relation to secondary bacterial pneumonia following influenza.

Recently, we reported findings showing that IAV infection triggers surface distribution of GP96 in human airway epithelial cells, where it is then hijacked as a host receptor for secondary infection by *S. pneumoniae* [39]. Although GP96 has been found mainly localized in the endoplasmic reticulum, abundant evidence presented indicates that it is also exposed on the surface of different cell types under particular conditions, such as infection, inflammation, cell activation, and necrotic cell death [40]. Surface-displayed GP96 is frequently exploited as a receptor for bacterial pathogens, including *Listeria monocytogenes* and *Neisseria gonorrhoeae* [41,42]. In the presence of *S. pneumoniae* infection, we identified the pneumococcal oligopeptide-binding lipoproteins AliA and AliB, which function as bacterial adhesins for GP96 on the surface of alveolar epithelial cells following an IAV infection. The oligo-binding proteins are also conserved among oral streptococci. Moreover, GP96 is a molecular chaperone that has a key role in folding, as well as surface expression of various integrin subunits and Toll-like receptors (TLRs) [43]. Notably, integrins are exported to the surface of IAV-infected cells

Table 1
Reported host receptors involved in enhancement of bacterial adherence to IAV-infected cells.

Stages	Receptors	Bacteria	Adhesins	Refs.
Inflamed				
	PAFR	*Streptococcus pneumoniae*	phosphorylcholine	[29]
		Non-typeable *Haemophilus influenzae*	phosphorylcholine	[30]
	pIgR	*Streptococcus pneumoniae*	CbpA	[36–38]
	GP96	*Streptococcus pneumoniae*	AliA, AliB	[39]
Healing				
	PLG, Fn	*Streptococcus pneumoniae*	PavB, PfbA,	[44,45]
	Fn, COL I, Lm	*Streptococcus pneumoniae*	RrgA	[46]
	Fn	*Streptococcus sanguinis*	PilA, PilB, PilC	[48]
	Fn, ITGα5β1, ITGαvβ3	*Porphyromonas gingivalis*	fimbriae	[49]
	Fn, Lm	*Prevotella intermedia*	AdpB	[50]
	Fn, Fgn	*Tannerella forsythia*	BspA	[51]

Abbreviations: PAFR, platelet-activating factor receptor; pIgR, polymeric immunoglobulin receptor; GP96, glycoprotein 96; *PLG, plasminogen; Fn, fibronectin; COL I, collagen I; Lm, laminin; ITG, integrin; Fgn, fibrinogen; CbpA,* choline-binding protein A; AliA & AliB, oligopeptide-binding lipoproteins; PavB, pneumococcal adherence and virulence factor B; PfbA, plasmin- and fibronectin-binding protein A; RrgA, pilus-associated adhesin; PilA, PilB & PilC, minor pilins; AdpB, broad-spectrum extracellular matrix-binding protein; BspA, basic surface-exposed protein A

in a GP96-dependent manner, thus extracellular GP96 and integrins promote bacterial colonization in airway epithelial cells. We have reported that treatment of IAV-infected mice with an GP96 inhibitor enhanced pneumococcal clearance from lung tissues and ameliorated pathological factors. It is thus considered that GP96 is a potential target for development of promising therapeutic strategies, including combination therapies, used as alternatives to conventional antibiotics and antiviral agents administered for broad-spectrum prevention, as well as management of secondary bacterial infections following influenza.

2.2.3. Injured or differentiating cells provide additional receptors

Secondary bacterial infections sometimes occur in patients who have begun to recover from the primary illness. In states of injury or cellular differentiation, the expression of apical receptors conducive to bacterial infection may be heightened. Areas characterized by incomplete healing, wherein basement membrane components, a thin layer of extracellular matrix (ECM), such as laminin or type I and type IV collagen, are exposed, or where fibrin and fibrinogen deposition has occurred, may contribute to a more pronounced bacterial adherence. In fact, the interaction between ECM components, including fibronectin, laminin, and collagens, and integrins functions as receptors for pneumococcal adherence to and invasion into host cells. [44–46].

Transforming growth factor β (TGF-β) plays a pivotal role in driving the regeneration process in response to inflammatory damage. During an IAV infection, viral NA has been shown to activate TGF-β, which, in turn, promotes the upregulation of host adhesion molecules, including fibronectin and integrins, ultimately leading to severe secondary bacterial pneumonia [47]. In addition to the bacterial pathogens responsible for secondary bacterial pneumonia, various oral bacteria, such as *Streptococcus sanguinis*, *P. gingivalis*, *P. intermedia*, and *Tannerella forsythia*, utilize interactions with fibronectin-integrins to colonize the oral mucosa. [48–51]. *P. gingivalis* infection has also been shown to promote TGF-β signaling [52]. Consequently, cytokine production from oral mucosa mediated by oral bacteria may modulate bacterial adherence to injured or differentiating airway epithelial cells and facilitate bacterial dissemination into the lower respiratory tract.

2.3. Epithelial damage and dysfunction

Airway epithelium is a specialized physical barrier that protects underlying sterile tissues from external contamination. Barrier integrity is generally maintained by a series of specialized complexes, including tight junctions, adherence junctions, and desmosomes. However, a dual viral-bacterial infection causes dysfunction of the epithelial-endothelial barrier, leading to exudation of fluids, erythrocytes, and leukocytes into alveolar spaces, thus causing gas exchange impairment and severe respiratory insufficiency. Indeed, pulmonary edema and hemorrhage are conditions commonly found in autopsy examinations [5]. The physical barrier function of airway epithelium is provided by four types of cell-cell junctions; tight, adherens, and gap junctions, and desmosomes.

2.3.1. Direct interaction of viral and bacterial factors with junctional proteins

Influenza virus infection-induced disruption of the pulmonary barrier has been associated with the loss of claudin-4 integrity, a tight junctional protein. [53]. In particular, interaction between the PDZ-binding motif of the avian influenza virus NS1 protein and PDZ domain present in tight junctional proteins has been demonstrated to destabilize epithelial junctional integrity [54]. Not only viral factors but also bacterial factors, such as pneumolysin, a cholesterol-dependent cytolysin produced by *S. pneumoniae*, has been shown to trigger the mislocalization and disruption of the adherens junctional protein E-cadherin in pulmonary cells [55]. *P. gingivalis* secretes arginine-specific and lysine-specific cysteine proteases, known as gingipains, which directly cleave the junctional protein JAM1 in gingival epithelial cells [56]. In co-infection with IAV, the synergy has been demonstrated to enhance the production of inflammatory cytokines and elevate nitric oxide (NO) production, resulting in heightened levels of apoptosis in lung epithelial cells [57]. The induction of apoptosis ultimately provides nutrients to invading opportunistic bacteria, following cytopathic damage and disruption of surfactant in the lungs. Hence, beyond viral infection, oral infections, particularly periodontitis, can induce modifications of cytokine production and epithelial barrier function, potentially increasing susceptibility to both viral and bacterial infections.

2.3.2. Signal transduction-mediated dysfunction

Multiple signaling pathways and their corresponding critical molecules play extensive roles in regulating the pathophysiological state of the barrier (Fig. 3). Calcium (Ca^{2+}) signaling has been implicated to be involved in various stages of host-pathogen interactions during viral and bacterial infections. Indeed, previous studies have reported that an IAV infection induces Ca^{2+} influx, then elevated intracellular Ca^{2+} promotes endocytic uptake of the virus, leading to a host inflammatory response [58,59]. Also, Ca^{2+} influxes activate calpains, Ca^{2+}-dependent host cysteine proteases, which then target junctional proteins such as occludin and E-cadherin in the airway mucosa [60]. In cases of *S. pyogenes* infection, calpains have been shown to be recruited to the plasma membrane along with E-cadherin [61]. In the case of co-infection with *S. oralis* and *Candida albicans*, calpain 1 activation results in oral mucosa dysfunction and subsequent systemic dissemination of *C. albicans* [62]. Notably, a preceding IAV infection draws calpains to the plasma membrane of paracellular junctions followed by destabilization of the airway epithelial barrier, which in turn promotes bacterial dissemination into deeper tissues [63].

IAV infection-mediated TGF-β signaling is not only critical for bacterial colonization of virus infected cells, but also causes dysfunction of the epithelial barrier. Activation of TGF-β signaling proceeds through phosphorylation of SMAD proteins, which is associated with Snail1-mediated down-regulation of tight junction proteins of epithelial and

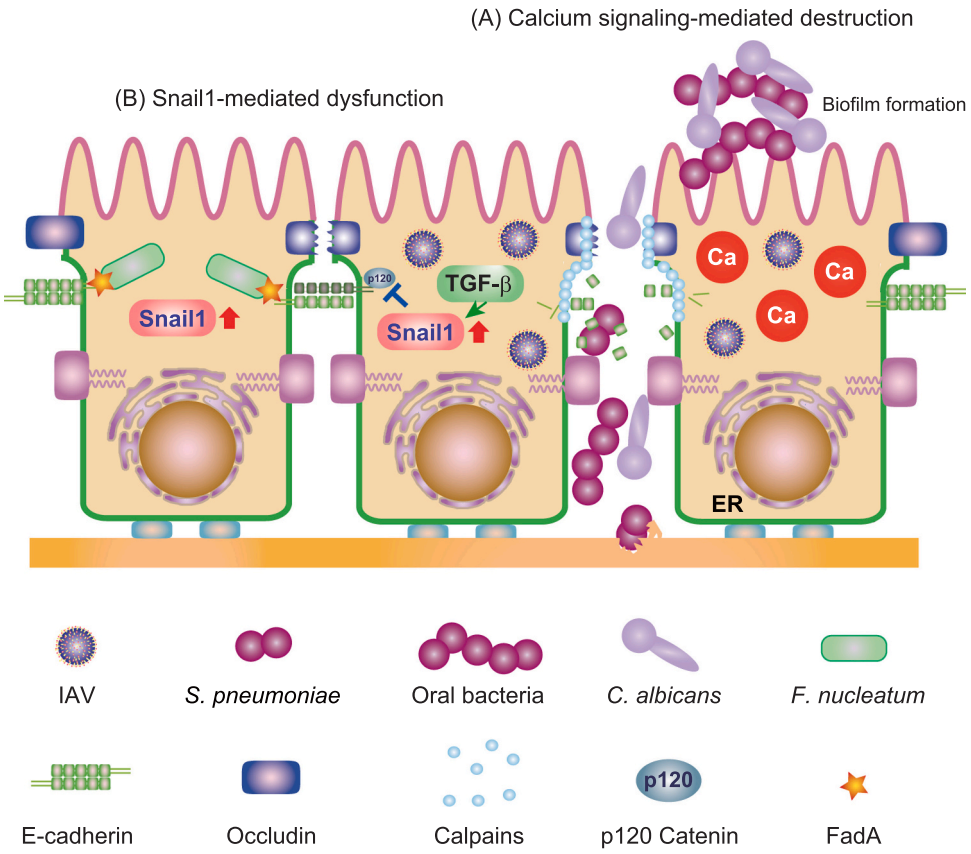

Fig. 3. Signal transduction-mediated dysfunction of airway barrier. Multiple signaling pathways and their corresponding critical molecules play extensive roles in regulating the pathophysiological state of the airway barrier. Respiratory tract pathogens have strategies to modify the signaling cascades, including (A) calcium signaling and (B) TGF-β signaling, leading to the dysfunction of the airway barrier and subsequent microbial translocation into deeper tissues via paracellular junctions.

endothelial cells. Our prior study provided evidence that a preceding influenza infection induces a Snail1-dependent dysfunction of the airway epithelial barrier through TGF-β signaling, thus preparing a route for secondary pneumococcal translocation into deeper tissues via paracellular junctions [39]. *F. nucleatum* also utilizes a surface adhesin, FadA, to bind to E-cadherin and activate Snail1 and β-catenin signaling, thereby regulating inflammatory and oncogenic responses [64,65]. While it remains unidentified whether the interaction between FadA and E-cadherin is crucial for the development of pneumonia at present, inflammation-induced airway tissue damage might contribute to an increased susceptibility to opportunistic bacterial pathogens.

3. Interaction between oral bacterial flora and viral infection

The oral cavity harbors the second-largest microbiota in the human body. Consequently, risk factors such as poor oral hygiene, increased inhalation, and viral infections create a pathway for oral microorganisms to enter the lower respiratory tract and potentially cause respiratory infections. Indeed, it has been speculated that the imbalance in nasopharyngeal microecology caused by the transcolonization of oral microbiota is associated with viral infections, leading to upper respiratory tract infections.

Viral infections have shown to drive alterations in both the local microbial composition and quantity in the lungs [66]. Dysbiosis in the respiratory tract, mediated by antiviral immune responses, can impact subsequent immune functions and inter-microbial interactions, potentially facilitating the proliferation of pathogenic bacterial species. Furthermore, the direct effects of the virus on the microbiome bacteria may facilitate the transition from pathobiont to pathogen. A metagenomic analysis of airway microbiotas from patients with the 2009 H1N1 pandemic revealed enrichment in genes related to cell motility, transcriptional regulation, metabolism, and response to chemotaxis compared to non-infected patients [67]. Another study noted that IAV infection had a significant impact on the *S. pneumoniae* transcriptome, downregulating genes associated with colonization and upregulating bacteriocins [68]. Thus, the direct effects of the virus on bacterial transcriptional patterns could be a mechanism by which colonizing bacteria gain invasive potential, ultimately leading to secondary bacterial pneumonia following influenza.

The influenza virus neuraminidase (NA) plays a crucial role in facilitating the release of virions from infected cells and promoting the spread of cell-to-cell infection. In addition to *S. pneumoniae*, *S. oralis* and *S. mitis* also produce bacterial NA with the ability to enhance viral proliferation [58,59]. Studies have demonstrated that the NA-specific inhibitor, zanamivir, is not effective against bacterial NAs from *S. pneumoniae*, *S. oralis*, and *S. mitis* [69,70], suggesting that the inhibitory effect of NA-specific inhibitors against the influenza virus might be attenuated by an increase in the number of NA-producing bacteria in the oral cavity. The oral bacterial flora serves as a major reservoir for respiratory infections. Therefore, considering the reduction of NA-producing bacterial flora through the improvement of oral hygiene might be a valuable approach to mitigate the risk of influenza pneumonia, as well as secondary bacterial pneumonia. On the other hand, we recently reported that *S. oralis* is capable of inactivating IAV through the production of short-chain fatty acids and hydrogen peroxide [71]. It appears that the synergistic mechanisms through which the oral bacterial flora can influence respiratory diseases, including influenza infection, are complex and multifactorial, simultaneously influenced by factors related to the oral environment, host, viral, and bacterial factors.

4. Viral infection-induced dysfunction of immune response to bacterial infection

A viral-bacterial co-infection undermines several aspects of mucosal immunity, with the primary result failure to regulate bacterial replication. Influenza virus infection specifically depletes airway-resident alveolar macrophages that are responsible for early bacterial clearance, which leads to deficits in early bacterial surveillance and subsequent killing. Indeed, IAV infection-induced depletion of alveolar macrophages (AMs) caused by promotion of apoptosis has been shown to facilitate bacterial superinfection [72,73]. In addition to reductions in cell numbers caused by depletion of AMs, the effects of IAV on AMs results in reduced production of cytokines and chemokines necessary for recruitment and activation of neutrophils, which can suppress NADPH oxidase-dependent phagocytic bacterial clearance, thereby enhancing susceptibility to secondary bacterial infection [74].

Dysregulation of proinflammatory cytokine response caused by a preceding virus infection is also generally believed to play a major role in predisposition to a secondary bacterial infection. Although the antiviral and immunostimulatory properties of type I IFNs have been well characterized, when IFN production is mistimed, inappropriate, and/or excessive, there can be detrimental effects. In addition to a preceding viral infection, the presence of oral bacteria, including *Prevotella*, and *Porphyromonas*, has the potential not only to modify the microbial composition of the respiratory system but also to initiate a sequence of cytokine responses, ultimately impacting the immune balance within the lungs. In fact, *Prevotella* primarily triggers TLR2 activation and amplifies the expression of inflammatory cytokines, including IL-23 and IL-1 [75]. Furthermore, *Prevotella* has been found to induce the production of IL-8, IL-6, and CCL20 in lung epithelial cells, thereby promoting a mucosal Th17 immune response and recruiting neutrophils. In the context of *P. gingivalis infection*, their proteolytic enzymes, known as gingipains, have been shown to be virulence factors contributing to pathological manifestations such as intrapulmonary hemorrhage, necrosis, and neutrophil infiltration in lung tissue. These manifestations correlate with systemic inflammatory responses, as evidenced by elevated levels of TNF, IL-6, IL-17, and C-reactive protein [76].

5. Conclusion

The ultimate goal of research related to viral-bacterial co-infections is to apply an improved understanding of the molecular mechanisms underlying these co-infections for the development of better diagnostic and treatment modalities, as well as prevention strategies. The interactions, particularly those involving influenza virus, the host, and *S. pneumoniae* as major bacterial respiratory pathogens, have been unveiled, as reviewed here. On the other hand, the specific host and bacterial factors that enable oral commensal bacteria to exploit virus infection-induced changes in airway barrier dysfunction and immune responses, leading to severe pneumonia in the elderly, have not been fully identified. Low-grade inflammation, referred to as 'inflammaging,' may also contribute to the pathogenesis of viral and bacterial co-infection. Periodontal disease is one of the most common chronic diseases, and its prevalence increases with age. We consider that age-associated inflammation, including periodontal disease, in concert with viral infection, enhances the expression of host receptors PAFR and GP96 in airway epithelial cells, thereby increasing the ability of avirulent bacteria, such as commensal oral bacteria, to associate with and invade the lower respiratory tract. Therefore, a better understanding of inflammaging-related changes to the barrier function of the respiratory tract during periodontal disease may lead to the establishment of effective therapeutic measures for the prevention of pneumonia in the elderly. Conversely, the elaboration of the acute inflammatory response during early infection has been found to decrease with age, resulting in a delayed immune response and diminished bacterial killing. Further research is needed to understand the role of inflammaging in relation to susceptibility and the severity of bacterial pneumonia following viral infection in elderly patients. Our goal is to broaden the research agenda for the underlying mechanisms of inflammaging-related pneumonia with a focus on the development of promising therapeutic measures.

The most crucial question is whether mechanisms elucidated in vitro or in animal models are genuinely significant in humans and have a substantial impact on actual epidemiology and pathogenesis. The recent development of multi-omics technologies, including genomics, proteomics, metabolomics, and single-cell transcriptomics, has enabled a fast and panoramic grasp of the pathogen and the disease. We consider that multiomics approaches, utilizing both animal models and humans, may have the potential to unravel the intricate mechanisms of viral and bacterial co-infections, including the transcriptome/epitranscriptome/proteome of the pathogens, virus–host-bacterial interactions, the immune landscape, inflammaging, and proteomic/metabolic biomarkers.

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Conflict of interest

None.

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