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Infectious and Inflammatory Pathways to Cough

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Abstract

Coughing is a dynamic physiological process resulting from input of vagal sensory neurons innervating the airways and perceived airway irritation. Although cough serves to protect and clear the airways, it can also be exploited by respiratory pathogens to facilitate disease transmission. Microbial components or infection-induced inflammatory mediators can directly interact with sensory nerve receptors to induce a cough response. Analysis of cough-generated aerosols and transmission studies have further demonstrated how infectious disease is spread through coughing. This review summarizes the neurophysiology of cough, cough induction by respiratory pathogens and inflammation, and cough-mediated disease transmission.

INTRODUCTION

Cough is a fundamental physiological response to irritation of the respiratory tract and serves to protect and clear the airway (1). Although cough can be voluntarily activated, it frequently occurs in otherwise healthy individuals as a hallmark involuntary symptom of respiratory infection (2, 3). Many viral, bacterial, and fungal respiratory pathogens have evolved virulence mechanisms to establish acute and chronic infections within the respiratory mucosa. These pathogens, or their microbial components, may interact directly with the complex pulmonary neuronal networks leading to cough (4–6). In addition to direct infection of pulmonary epithelia and immune cells, host immune responses to infection result in significant cellular infiltration and release of inflammatory signals. Substantial influx of inflammatory cytokines such as interferons (IFNs), tumor necrosis factor alpha (TNF-α), interleukin (IL) 1β (IL-1β), and lipid/peptide mediators (bradykinin, leukotrienes) has also been linked to induction of cough or airway sensitization (7). These accompanying inflammatory responses may also work independently or in concert with microbial components to regulate the infectious cough response. The resultant activation of productive cough further aids in the generation of microbe-containing aerosol particles as a mode of disease transmission (8). Cough dynamics and aerosol transmission studies have characterized the critical role of cough in spreading disease and how donning preventative protective equipment (i.e., face masks) mitigates infection (9). Although it is evident that respiratory infections lead to cough, the conserved or unique mechanisms by which pathogens cause cough remain largely undefined and a current focus of the field. This review summarizes the present knowledge of the underlying neurophysiological pathways leading to cough and induction of cough by common respiratory viral, bacterial, and fungal pathogens. Additionally, the inflammatory mechanisms of cough and the impact of coughing on disease transmission are discussed.

THE NEUROPHYSIOLOGY OF COUGH

Coughing functions to clear the respiratory tract of irritants and excess mucous and is characterized by a forcible expulsion of air from the lungs with a characteristic sound. However, the underlying physiology of how cough is generated is more complex (10). There are three distinct phases to a cough event that distinguish cough from other respiratory responses (**Figure 1**). Cough begins with the inspiratory phase, where inhalation of air serves to lengthen expiratory muscles. The next phase, the compressive phase, involves a brief closure of the glottis to sustain lung volume during initial contraction of expiratory muscles such that intrathoracic pressure increases sharply (1, 10). Finally, the expiratory phase begins by the opening of the glottis to release expiratory flow followed by 200–500 ms of lower expiratory flows. The resultant dynamic compression of the airways and high velocity air flow generate the characteristic cough sound and promote mucociliary clearance (11).

Cough motor patterning involves reconfiguration of the normal brainstem respiratory circuit activity, and this can be initiated either reflexively via sensory inputs from the airways and lungs or volitionally via descending pathways arising from the motor cortex (12). Additionally, the induction of cough is usually associated with the perception of an urge to cough, a higher brain-encoded sensation representative of airway irritation and accompanied by alterations in emotive and cognitive processing. These higher-order processes help shape the volitional control of cough and contribute to the overall conscious awareness of cough-evoking stimuli (13, 14). Thus, while the act of coughing is often considered a motor reflex, the underlying neurophysiology is highly complex and dependent on activity at all levels of the neuroaxis.

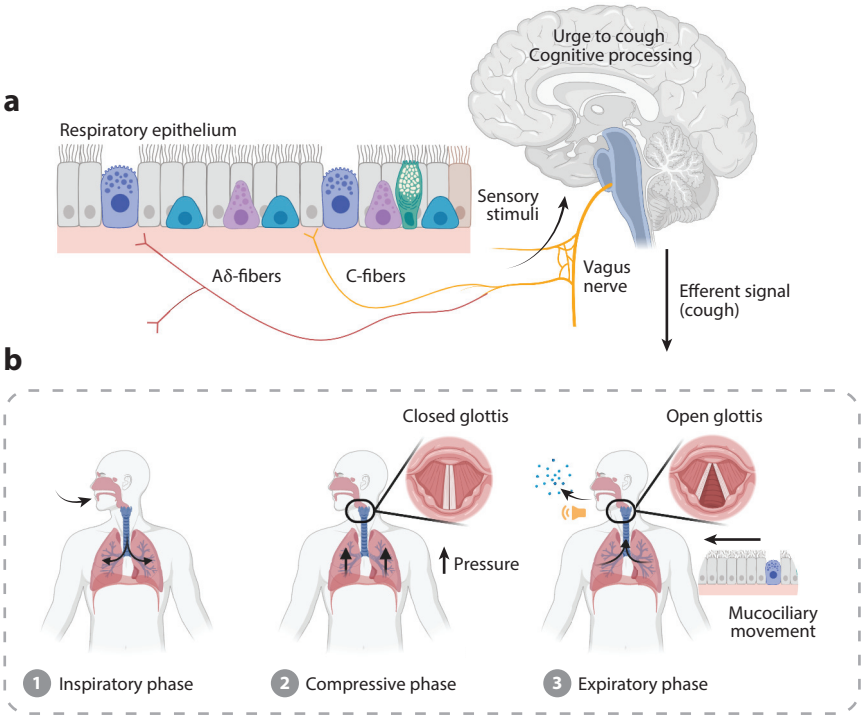

Figure 1

Cough physiology. (*a*) Airway sensory neurons innervate the respiratory epithelium and respond to irritant stimuli present in the respiratory tract. Sensory signals are carried through the vagus nerve to the brainstem where an urge to cough is encoded. The resulting cognitive processing initiates the cough event. (*b*) Cough begins with ① inhalation of air and lengthening of expiratory muscles in the inspiratory phase. Next, ② the compressive phase involves a brief closure of the glottis to increase intrathoracic pressure. Finally, ③ in the expiratory phase, the opening of the glottis releases air at a high velocity, producing the characteristic cough sound and facilitating mucociliary movement. Figure adapted from images created with BioRender.com.

Cough Sensory Neurons

Airway vagal sensory neurons arise from the nodose and jugular ganglia and have distinct phenotypes and functions (2, 15, 16). A major distinguishing factor between jugular and nodose neurons is their origin of embryonic development. Airway jugular neurons, derived from the neural crest, function similarly to the somatosensory spinal nerves to sense noxious chemical and thermal stimuli (15). The nodose neurons, however, are derived from cells within the placodes, and although a subset of airway nodose neurons detect noxious stimuli, many nodose neurons survey the physiological state of visceral organs, including the airways and lungs (15). The complex neurobiology of airway vagal sensory pathways and their role in pathological conditions have been comprehensively reviewed (2). Briefly, sensory nerve terminals originating from the nodose and jugular ganglia are distributed throughout the airway in close association with the airway epithelium, the airway smooth muscle, and vasculature and glandular tissues. Functionally, airway neurons are broadly divided into main groups based on either their speed of action potential conduction: C-fibers, Aδ-fibers, and Aβ-fibers; or their physiological sensitivity: chemically sensitive afferents and mechanically sensitive afferents. Chemically sensitive afferents conduct action potentials

Nodose ganglia: inferior ganglia of the vagus nerve below the jugular foramen, derived from the placodes

Jugular ganglia: superior ganglia of the vagus nerve as it traverses the jugular foramen, derived from the neural crest

in the range of C- and Aδ-fibers and are commonly referred to as airway nociceptors, as they largely respond to noxious chemical stimuli such as capsaicin, bradykinin, or sulfur dioxide (17, 18). Mechanically sensitive afferents can be Aδ- or Aβ-fibers and include fibers important for the physiological control of respiratory function, such as the rapidly and slowly adapting stretch receptors involved in the Hering-Breuer deflation and inflation reflexes (3, 19). A specialized subset of rapidly adapting Aδ-fibers are mechanoreceptors (sometimes called the cough receptor subtype) that also respond to noxious mechanical and acidic stimuli, such as those with the inhalation of particulate matter, aspiration of foodstuffs or gastric contents, and the accumulation of airway mucous (20). Further subtypes of these major afferent populations can be defined based on their terminal distributions in the bronchial or pulmonary airways (21, 22), their unique physiological responsiveness (18), and their differing molecular phenotypes (23, 24).

C-fibers: unmyelinated peripheral nerve fibers responsible for transmitting noxious signals at a low conduction velocity

Aδ-fibers: thinly myelinated nerve fibers that respond to temperature, pressure, and chemical stimulation and send impulses faster than unmyelinated fibers

Aβ-fibers: myelinated mechanoreceptors that transmit sensory signals at a high conduction velocity

Cough receptor: neuron that innervates the larynx, trachea, and bronchi and responds to mechanical and acidic stimulation to initiate coughing

Ionotropic receptors: protein receptors that form a ligand-gated ion channel such that binding of a ligand allows ions to flow through it

Metabotropic receptors: membrane receptors that initiate an intracellular cascade upon ligand binding; also referred to as G protein–coupled receptors

Among the array of distinct airway vagal sensory neurons identified, two specific subtypes are believed to be important for the induction of coughing, namely the nodose-derived Aδ-fibers cough receptors and the nociceptive C-fibers (especially those derived from the jugular ganglia) (20, 25). Collectively, these two types of cough-evoking sensory neurons encode responsivity to a wide range of physical and chemical irritant stimuli that may reach the airway mucosa via inhalation, aspiration, or endogenous production. Both pathways are therefore important for protecting the airways and lungs in health and, similarly, both pathways are believed to be important contributors to excessive cough characteristic of many pathological conditions.

Sensory inputs from vagal neurons are translated into motor output, to generate cough, through processing by the brainstem (26). Studies in animals and humans suggest that primary cough-related sensory neurons terminate onto second-order neurons in the nucleus of the solitary tract and paratrigeminal nucleus, which project to the brainstem respiratory circuits (26–29) to encode the cough motor pattern. Vagal sensory information can ascend from the brainstem to the cerebral cortex, through central nervous system networks that are essential for the generation of an urge to cough and voluntary cough suppression or induction (7, 30). Additionally, plasticity in central neuronal processing can alter the nature of the output and contribute to cough hypersensitivities (31). Although the mechanisms are not fully characterized, plasticity of cough neural circuits can have implications in chronic cough and inflammatory diseases (32).

Cough Sensory Transduction Mechanisms

Cough-evoking stimuli interact with proteinaceous receptors present on vagal afferent nerves to initiate, or lower the threshold for, action potential discharge (2). The receptors involved in transducing cough stimuli can be broadly categorized as ligand-gated ionotropic receptors or transmembrane metabotropic receptors coupled to G proteins [G protein–coupled receptors (GPCRs)] or other intracellular signaling pathways (**Figure 2**).

The transient receptor potential (TRP) channels comprise a large family of ion channel receptors on respiratory sensory neurons. TRP vanilloid 1 (TRPV1) is a member of the TRP family and functions as a nonselective cation channel for calcium and sodium influx (33). Capsaicin, a strong activator of most chemically sensitive vagal C-fibers, activates TRPV1 through the vanilloid moiety to stimulate neuron activation (34, 35). TRPV1 gating is also induced or potentiated by heat, including during hyperthermia (36) and by stimuli acting at several GPCRs (37). TRPV1-expressing sensory neurons often coexpress one or more of the other TRP ion channel family members, including TRP subfamily A member 1 (TRPA1) and TRP subfamily M member 8 (TRPM8) (38). Agonists of TRPA1, such as allyl isothiocyanate and acrolein, lead to cough induction in guinea pigs (39, 40) and synergistically interact with activators of TRPV1 to promote C-fiber activation (41). TRPM8 is activated by menthol and thermal sensation in the cool to cold range (42, 43) and typically leads to suppression of coughing (44, 45). Inhalation or aspiration of

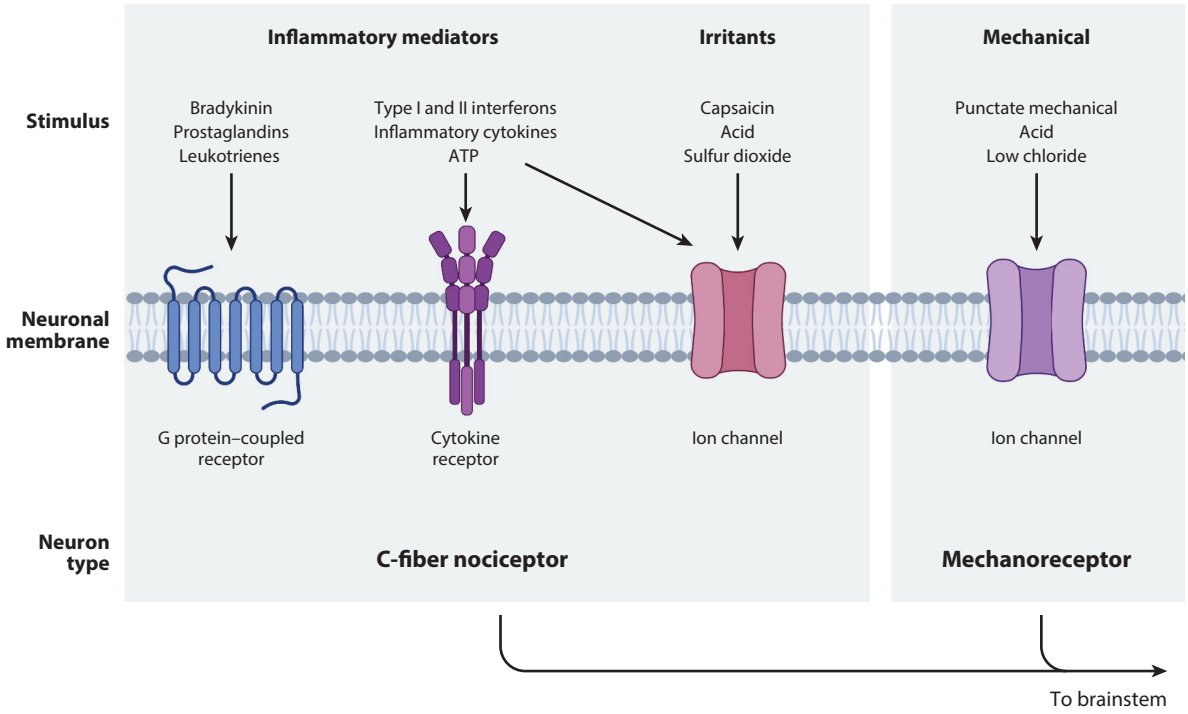

Figure 2

Mechanisms of cough activation. Mechanical or chemical stimulants present in the airways act through neuronal membrane receptors to initiate a cough response. Inflammatory mediators (cytokines, ATP, peptide, and lipid mediators) act through G protein–coupled receptors, cytokine receptors, and ion channels present on C-fiber nociceptive neurons. Chemical irritants such as capsaicin, acid, or sulfur dioxide act through ion channels to activate C-fibers, which carry sensory information to the brainstem. Mechanical stimulation acts through ion channels present on mechanoreceptors, which signal to the brainstem. Abbreviation: ATP, adenosine triphosphate. Figure adapted from images created with BioRender.com.

acidic solutions or acidification of endogenous compounds can also lead to chemical activation of cough-evoking neurons (46, 47). Although there is evidence for acid-induced activation of C-fibers through TRPV1 (48, 49), acidic conditions can also activate airway Aδ-fiber cough receptors that do not express TRPV1 (50). Nodose and jugular neurons express the acid-sensing ion channel (ASIC) family of channels that recognize rapid decreases in pH (51), including ASIC1, ASIC2, and ASIC3, leading to acid-mediated activation of nodose Aδ-fiber cough receptors (46). An important role for ionotropic purinergic signaling has been identified in airway vagal neurons involved in cough generation and sensitization (52). Adenosine triphosphate (ATP), released by airway epithelia or other resident cells (53, 54), activates airway vagal afferents via purinergic receptors (P2X) comprised of homotrimeric P2X3 subunits or heterotrimeric P2X2/3 subunits (52). Indeed, inhaled ATP alone triggers cough in animals and humans through P2X3- or P2X2/3-dependent pathways (28, 55). Many of these ionotropic transduction processes have been explored clinically for their role in excessive cough accompanying disease (56).

GPCRs are seven-transmembrane-helix proteins coupled to intracellular signaling G proteins (57). Agonists of GPCRs can impact the sensitivity of vagal C-fibers to capsaicin (58). A commonly used activator of C-fibers, bradykinin, can stimulate action potential in canine airways (59), and mouse studies demonstrate that bradykinin-induced cough is inhibited by TRPV1 antagonists (49). Bradykinin also activates beta-2 (B2) adrenergic receptors on jugular and nodose

C-fibers, leading to parasympathetic reflexes and coughing (60). The lipid mediators, leukotrienes and prostaglandins, can also modulate neural activation by stimulating GPCRs. Prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2) lead to reflex bronchoconstriction in canines (61), and cough stimulated by PGE2 in guinea pigs is attenuated by prostaglandin E receptor 3 (EP3) receptor antagonists (62). These findings demonstrate that inflammatory mediators can induce cough through GPCR-mediated mechanisms.

The interferon class of receptors recognize the cytokines IFN-α, IFN-β, and IFN-γ that are frequently secreted when immune cells encounter infectious pathogens. These receptors are also expressed on vagal C-fiber neurons and can be activated to initiate cough and other defensive reflexes. Administration of IFN-γ enhances the guinea pig cough response to citric acid and increases phosphorylation of the downstream IFN-γ receptor signaling molecule signal transducer and activator of transcription 1 (STAT1) in vagal sensory nerves (63). Similarly, IFN-γ is significantly increased in sputum from patients with chronic refractory cough and inhalation of IFN-γ prior to capsaicin exposure increases cough sensitivity (64). In addition to IFN-γ, type I IFNs activate vagal sensory nerves, and the type I IFN receptors are highly expressed on TRPV1-positive neurons from mouse lungs (65). The emerging roles of IFNs and IFN receptors in neural activation are of particular importance in the context of pathogen-induced cough, as IFNs are abundantly produced during airway infections (66, 67).

COUGH INDUCTION BY RESPIRATORY PATHOGENS

In otherwise healthy individuals, acute cough is most often associated with respiratory tract infections. Although cough is a common symptom shared by many respiratory diseases, the mechanisms by which viral, bacterial, and fungal airway pathogens may directly or indirectly induce cough are a growing area of research (**Figure 3**). Whether cough has evolved to protect the host from infection or has been leveraged by airway pathogens to facilitate survival, improve replication, and/or enhance transmission remains a critical question. Because airway pathogens have evolved a variety of virulence mechanisms to establish acute and chronic infections within the mucosa of the lung, it also seems feasible that they would evolve mechanisms to facilitate transmission by evoking a cough or sneeze reflex. In addition, while infections can cause extensive lung inflammation and excessive mucous production, which can also induce cough, interactions between pathogens and cough-inducing neurons or receptors could also be direct cough triggers.

Viruses

Upper respiratory tract infections are a major cause of morbidity across the world, and cough is the most frequent symptom associated with disease. The most common etiologies responsible for upper respiratory tract infections are viral pathogens from the picornavirus and coronavirus families but can also include influenza viruses and pneumoviruses such as human metapneumovirus and respiratory syncytial virus (RSV), among others (68). Infection of epithelial and neuronal cells in vitro with respiratory viruses results in significant upregulation of ion channels such as TRPV1, TRPA1, and ASIC3. In neuroblastoma cells, rhinovirus triggers upregulation of TRPA1 and TRPV1 by either replicating virus or ultraviolet-inactivated viral preparations, while TRPM8 requires live virus for messenger ribonucleic acid (mRNA) expression (68). Similarly, in vitro infection of human neuroblastoma cells with measles virus and RSV induces upregulation of TRPV1 and ASIC3 (69). Although receptor expression is upregulated during viral infection, ultraviolet-inactivated viral preparations and virus-induced soluble factors are also sufficient to upregulate TRPV1 and ASIC3 mRNA, suggesting that proteins, lipids, or small molecules associated with viral particles may be sufficient to stimulate cough (69). In addition to in vitro studies, infectious

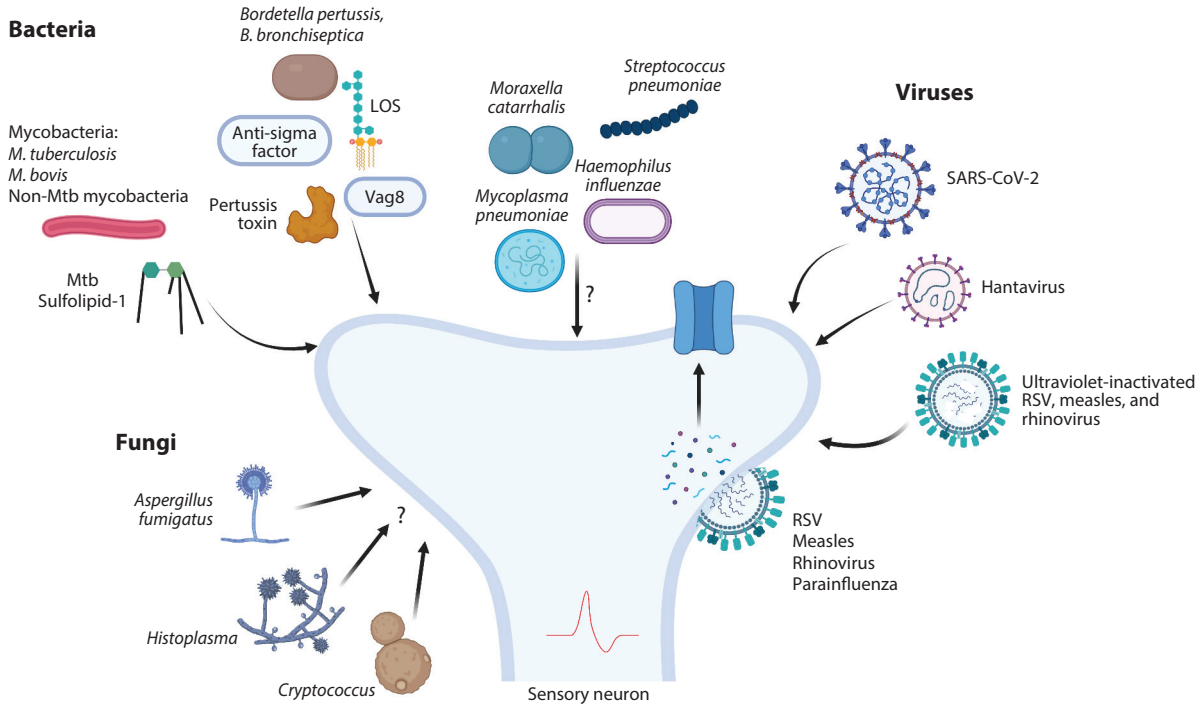

Figure 3

Pathogen activation of cough. Respiratory pathogens activate a cough response through proteins, lipids, or small molecules. Infection with Mtb leads to nociceptor and cough activation through the glycolipid sulfolipid-1. Other mycobacteria, including *Mycobacterium bovis* and nontuberculosis mycobacteria, also initiate cough through shared or distinct pathways with Mtb. The bacterial pathogen *Bordetella pertussis* produces pertussis toxin, LOS, and Vag8, which cooperatively initiate paroxysmal cough. Another *Bordetella* species, *B. bronchiseptica*, produces the anti-sigma factor BspR/BtrA to regulate cough. Infection with other bacterial pathogens, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, and *Haemophilus influenzae*, results in cough through unknown cough-inducing molecules. Fungal pathogens *Aspergillus fumigatus*, *Histoplasma*, and *Cryptococcus* produce similar symptoms to common bacterial infection, including cough, through unknown fungal compounds. Viral infection leads to production of cough and severe respiratory disease (e.g., SARS-CoV-2, hantavirus). Infection with live and ultraviolet-inactivated viruses (e.g., RSV, measles, rhinovirus, and parainfluenza) results in the upregulation of ion channels that activate the cough response. Abbreviations: BspR/BtrA, *Bordetella*-secreted protein regulator; LOS, lipooligosaccharide; Mtb, *Mycobacterium tuberculosis*; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Vag8, virulence-associated gene 8. Figure adapted from images created with BioRender.com.

disease models of cough have been used to further identify the influence of respiratory pathogens on afferent neurons. Parainfluenza virus type 3 (PIV3) infection of guinea pigs leads to significantly increased sensitivity to the cough-inducing agonists capsaicin, citric acid, and bradykinin. As with measles and RSV, PIV3 infection leads to increased expression of TRPV1 in tracheal nodose and jugular C-fiber neurons in inoculated animals (4). Capsaicin-induced airway relaxation is also affected by influenza A infection, likely through reduction of PGE2 generation (70). In addition to viruses with pandemic potential like influenza that can engage the airway nervous system, the emerging zoonotic virus from the Bunyaviridae family, known as hantavirus, can lead to severe and fatal hantavirus cardiopulmonary syndrome when individuals inhale rodent excrement (71). Severe cases are associated with elevated serum inflammatory cytokines (including IFN-γ) and prominent symptoms of cough, dyspnea, and hypoxia (71, 72). These data provide potential mechanisms by which respiratory viruses may directly, or indirectly, induce airway hypersensitization and cough (4, 68, 69).

Much like other, less-severe respiratory viruses, cough is a key symptom of severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) infection that persists into the postinfectious phase of disease (73). While cough has carried stigmatization of disease throughout history, the effects of social isolation related to cough were heightened during the coronavirus disease 2019 (COVID-19) pandemic. Many research efforts have been underway to understand the pathogenic mechanisms of SARS-CoV-2, and cough induction has been no exception. Although direct evidence for SARS-CoV-2-induced cough has yet to be established, there are many proposed mechanisms for COVID-19-related cough given the interplay between infection and sensory nerve dysfunction (74). In addition to cough, symptoms of SARS-CoV-2 infection include sensory dysfunction related to olfactory and taste impairment (75). While vagal sensory neurons do not readily express the primary entry receptor for SARS-CoV-2, angiotensin-converting enzyme 2 (76), additional entry factors may allow for direct infection of vagal sensory neurons (77). SARS-CoV-2 may infect neurons, but there is more evidence for viral entry into vascular and immune cells which cause local inflammatory responses. This local inflammation may also contribute to respiratory hypersensitivity or impact brainstem regions responsible for respiratory control, thus leading to prolonged cough (75, 78). Additionally, targeting TRP channels has been proposed to ameliorate many neurological symptoms associated with COVID-19 (79). Direct mechanisms by which SARS-CoV-2 may induce cough remain to be elucidated, but the implications of infection on neuronal function suggest both indirect and direct viral interaction with airway neurons leading to cough even after many disease symptoms have subsided.

Bacteria

Many bacterial organisms can also establish infection in the airways, leading to acute and persistent cough. A 2003 study found that patients with recurrent bacterial pneumonia, defined by at least two episodes of pneumonia in one year that responded to antibiotics, experienced increased sensitivity to capsaicin-induced cough (80). In pediatric patients, chronic wet cough is a common symptom of protracted bacterial bronchitis (PBB) (81). Bronchoalveolar lavage of subjects with PBB most often contain *Haemophilus influenzae*, while *Streptococcus pneumoniae* and *Moraxella catarrhalis* can also cause PBB (82). Prolonged wet cough even after antibiotic treatment can further increase likelihood of bronchiectasis in PBB patients, although the mechanisms by which cough is sustained are unknown (83). *Mycoplasma pneumoniae* infections lead to refractory or severe pneumonia and cough, especially in children (84). Lipid-associated membrane proteins from *M. pneumoniae* lead to release of inflammatory cytokines including high-mobility group box protein 1, TNF-α, and IL-6 through Toll-like receptor 2 signaling (85).

The bacterial pathogen *Bordetella pertussis* causes severe infection of the respiratory tract, leading to paroxysmal cough in infants, otherwise known as whooping cough, owing to the characteristic whoop sound made at the end of a cough paroxysm. Though *B. pertussis* produces numerous virulence factors including pertussis toxin, adenylate cyclase toxin, and heat-labile toxin, among others, a cough toxin has yet to be fully identified. Rats infected by intrabronchial delivery with agarose-encased *B. pertussis* develop respiratory paroxysms resembling cough (86). Induction of cough following *B. pertussis* infection was also demonstrated in a nonhuman primate model where 100% of infected olive baboons developed clinical pertussis (87). Interestingly, when *B. pertussis* strains lacking pertussis toxin are administered to rats, animals exhibit reduced or no coughing compared to strains that produce pertussis toxin (88). Similarly, in the baboon model, maternal vaccination using a monocomponent pertussis toxoid vaccine confers protection against clinical symptoms of severe pertussis in infants (89). These studies suggest a correlation between *B. pertussis* infection and coughing and highlight the important role of pertussis toxin in cough induction. To further assess the mechanisms by which *Bordetella* species cause cough, a rat model

of *B. bronchiseptica* infection was employed. Although pertussis toxin was a probable candidate for *Bordetella*-induced cough, a *B. bronchiseptica* deletion mutant in the *ptx-ptl* gene produces cough similar to wild-type *B. bronchiseptica*. Pertussis toxin from *B. bronchiseptica* is not sufficient to induce cough, although the anti-sigma factor BspR/BtrA regulates cough in infected rats (90). While many of these earlier studies relied on sound recordings for cough measurements, recent efforts have been made to establish a rat model for *B. pertussis* cough detection using whole body plethysmography. Rats infected with the D420 strain of *B. pertussis* exhibited significantly increased cough events compared to mock-challenged rats or those infected with the Tohama 1 strain, which has lower expression of pertussis and adenylate cyclase toxins (91). In this new coughing rat model, mucosal vaccination with an acellular pertussis vaccine significantly reduced cough post-challenge in vaccinated animals (92). Furthermore, specific cough-inducing factors of *B. pertussis* include lipooligosaccharide, pertussis toxin, and virulence-associated gene 8 (Vag8), which cooperatively function to induce cough in mice based on audio and visual recordings (93). These results highlight the complexity of cough induction by *Bordetella* spp. and suggest that there may be distinct cough pathways that depend on the unique *Bordetella* spp. substrains associated with respiratory disease and the specific animal host.

Among the symptoms of pulmonary tuberculosis caused by *Mycobacterium tuberculosis* (Mtb) infection, chronic or bloody cough (hemoptysis) is a hallmark of disease and a critical mechanism of disease transmission (94–96). Due to the highly inflammatory nature of tuberculosis, a prevailing hypothesis in the field suggested that inflammatory mediators were the primary mechanism by which cough was induced during active disease (97). However, Ruhl et al. (5) identified that organic extract from Mtb activates nociceptive neurons and induces cough in a guinea pig model. Furthermore, the cell wall glycolipid sulfolipid-1 (SL-1) was identified to be a cough-inducing molecule produced by Mtb. Through deletion of genes in the SL-1 synthesis pathway, Mtb extracts from mutant strains are unable to induce cough or neuron activation, while complementation restores the SL-1 phenotype independent of inflammatory influx (5). Thus, Mtb plays a direct role in inducing cough and neuronal activation, providing a basis for identifying a putative SL-1 cough receptor and determining the role of SL-1-induced cough in the transmission of infectious particles. Interestingly, the pulmonary disease tuberculosis can also be caused by *Mycobacterium bovis*, a member of the Mtb complex. However, human-to-human spread of *M. bovis* has been only rarely observed, suggesting that *M. bovis* may not be as transmissible as Mtb, and data on cough in the setting of *M. bovis*–associated human tuberculosis are lacking. Most humans are infected by zoonotic exposure to *M. bovis*, including by gastrointestinal infection from unpasteurized dairy products, cutaneous transmission from contact with infected wounds, exposure to animal airway secretions, and possible airborne contact (98). Of note, the predominant *M. bovis* strain that causes bovine tuberculosis and rare cases of human tuberculosis does not produce SL-1 (99). A key Mtb and *M. bovis* signaling pathway for SL-1 production is the two-component system PhoP/PhoR (100, 101). *PhoP* is under positive natural selection in Mtb and is proposed to be critical for transmission (102, 103). In contrast, most *M. bovis* strains encode a mutant *phoP* allele, accounting for the lack of SL-1 production (99). However, an *M. bovis* strain responsible for a tuberculosis outbreak in HIV-infected patients (104, 105) was found to contain an *IS6110* insertion in the *phoP* promoter (106) that restored expression of the PhoP/PhoR regulon and SL-1 production (99). Thus, SL-1 expression is associated with Mtb and *M. bovis* transmission.

Although Mtb causes the most severe pulmonary disease and is responsible for the most morbidity and mortality, human infection with nontuberculous mycobacterial (NTM) species also causes pulmonary disease. NTM infection manifests with chronic cough, particularly in individuals with altered pulmonary immunity (i.e., due to HIV/AIDS, organ transplantation, or cystic fibrosis) or structural defects like bronchiectasis. The primary species isolated from those with

NTM-related pulmonary disease include *Mycobacterium avium-intracellulare* complex, *M. xenopi*, *M. kansasii*, and *M. abscessus* complex (107). These organisms do not produce SL-1, suggesting that other molecules produced by these species may function as nociceptive agonists.

Postinfectious cough: coughing that persists 3–8 weeks after the onset of upper respiratory infection and in the absence of other defined causes

Fungi

Although the evidence for viral and bacterial-induced cough continues to grow, fungal-associated acute and chronic cough remains largely understudied. A clinical study enrolled patients with chronic cough and who also had sputum cultures positive for basidiomycetous fungi to test the role of low-dose antifungal treatment on cough. Compared to the placebo group, subjects treated with itraconazole had significantly reduced levels of cough using a subjective cough symptom scale (108). This finding led to a proposed clinical description of fungus-associated chronic cough using the following criteria: chronic cough, presence of fungi in the sputum, and a clinical response to antifungal drugs. Further clinical studies from the same group described sensitization to *Bjerkandera adusta* in chronic intractable cough (109, 110). Treatment with low-dose itraconazole was inconclusive in relieving chronic idiopathic cough but may have implications in allergic fungal cough (111).

Pulmonary aspergillosis, caused primarily by the *Aspergillus fumigatus* complex, mimics many symptoms of pulmonary tuberculosis, including fever, chills, weight loss, cough, and hemoptysis, and occurs in individuals with congenital or acquired immunodeficiency (112). Beyond aspergillosis, individuals with weakened cell-mediated immunity (i.e., HIV/AIDS, organ transplantation, chronic steroid use) are at high risk for severe fungal pulmonary infections. For example, *Pneumocystis jirovecii* is a fungal pathogen responsible for life-threatening interstitial pneumonia primarily in immunocompromised individuals that manifests with insidious symptoms of dry cough, fever, and dyspnea over days to weeks (113). Because cough is a nonspecific symptom of respiratory infection shared among viral, bacterial, and fungal pathogens, it can sometimes be challenging to identify a precise etiology. Analysis of symptom records from adult outpatients shows that those with blastomycosis, coccidioidomycosis, cryptococcosis, and histoplasmosis are often misdiagnosed with community-acquired pneumonia, influenza, tuberculosis, or lung cancer due to overlapping symptomology (114, 115). Thus, pulmonary fungal infection may be a more common cause of symptomatic cough for which people seek medical attention than previously recognized, and may, like with pertussis and tuberculosis, be triggered by nociceptive neuron activation from pathogenic molecules.

INFLAMMATORY MECHANISMS IN COUGH

Respiratory infections cause substantial lung inflammation, exposing the respiratory tract to cytokines, chemokines, and lipid mediators (7) (**Figure 4**). These inflammatory mediators can act as agonists for cough-inducing receptors and interact with the dense network of nociceptive neurons lining the airways (2). Therefore, in addition to direct activation by pathogens, infectious disease-related cough can also be a product of the inflammatory microenvironment initiated by host protective mechanisms.

Virus-induced lung damage can be triggered by exuberant inflammation and cytokine release, which may contribute to cough hypersensitivity. Patients with postinfectious cough following acute respiratory tract infections have increased sputum eosinophilia and airway inflammation (116). Additionally, accumulation of lymphocytes in bronchoalveolar lavage fluid is associated with patients who report chronic cough (63). These lymphocytes, primarily T cells, produce IFN-γ in response to infection. IFN-γ triggers cough in patients with idiopathic pulmonary fibrosis and exposure of guinea pigs to exogenous IFN-γ enhances their cough response to citric acid (63). In an in vitro model of vagal sensory neurons, IFN-γ induces calcium influx and action potentials in

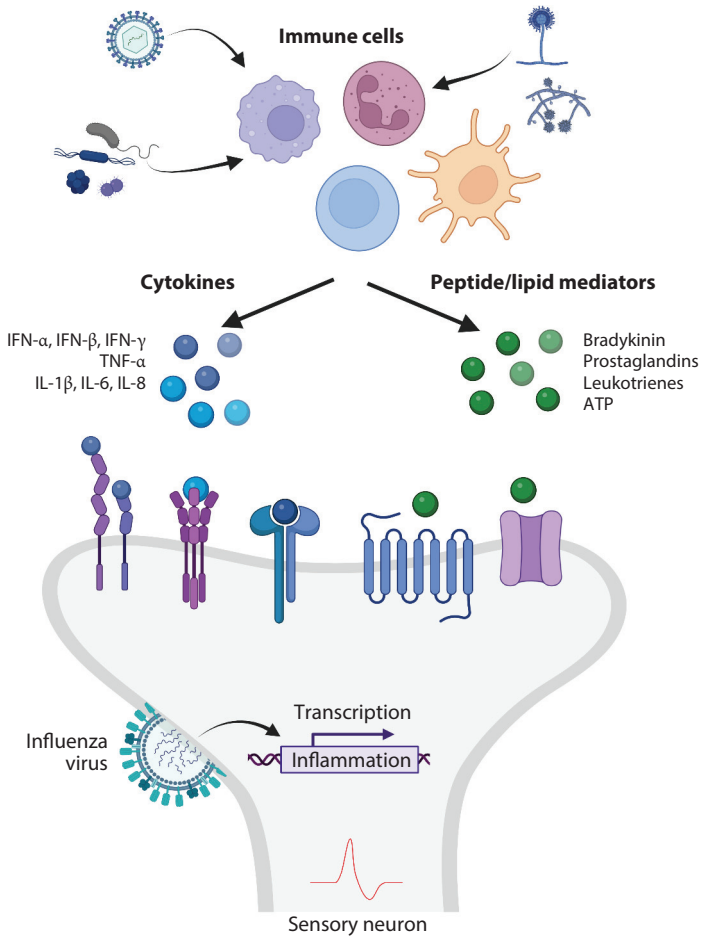

Figure 4

Inflammatory mediators and cough. Immune cells (e.g., macrophages, neutrophils, dendritic cells, and lymphocytes) of the respiratory tract are activated or directly infected by bacterial, viral, and fungal pathogens. In response to infection, immune cells secrete inflammatory cytokines (IFN-α, IFN-β, IFN-γ, TNF-α, IL-1β, IL-6, and IL-8) and peptide or lipid mediators (bradykinin, prostaglandins, ATP, and leukotrienes). Inflammatory molecules bind to cytokine receptors, G protein–coupled receptors, or ion channels on sensory neurons to activate a cough response. Additionally, respiratory pathogens may directly infect neurons to induce vagal neuroinflammation. Abbreviations: ATP, adenosine triphosphate; IFN, interferon; IL, interleukin; TNF-α, tumor necrosis factor alpha. Figure adapted from images created with BioRender.com.

a JAK/STAT signaling pathway-dependent manner (63). While direct infections were not tested, the implications of IFN-γ-mediated cough induction can be extended to several bacterial and viral pathogens associated with increased T cell infiltrate in the lung during infection (97, 117). In addition to IFN-γ, experimental inoculation of rhinovirus 16 increases IL-8 in nasal lavage and airway hypersensitivity to histamine (118). The proinflammatory cytokines TNF-α and IL-1β can also stimulate vagal nerves, further demonstrating the impact of pulmonary inflammation on airway hypersensitivity (119, 120).

Autacoid: locally produced and expressed factor that affects physiology and is not part of traditional immune or autonomic groups

Bradykinin is a peptide autacoid formed from multifunctional precursor glycoproteins called kininogens that is released during various pathological conditions. In an unanesthetized guinea

pig model of cough, bradykinin administration activates cough through B2 receptors on bronchopulmonary C-fibers (121). In addition to B2 receptors, antagonists of the TRPV1 and TRPA1 receptors inhibit bradykinin-dependent cough hypersensitivity (60). Bradykinin-mediated activation has been hypothesized as a mechanism for paroxysmal coughing during *B. pertussis* infection (122). Furthermore, paroxysmal coughs are a shared phenotype in guinea pigs administered bradykinin and infected with *B. pertussis* (121). Lipooligosaccharide and Vag8, cough-inducing factors of *B. pertussis* in mice, activate bradykinin production, leading to sensitization of the TRPV1 receptor (93).

Neuropathy: damage or dysfunction of peripheral nerves resulting in pain, numbness, or weakness

Although many inflammatory mediators can signal through ion channels to trigger cough, nociceptive neurons also regulate protective immune responses. In a lethal model of *Staphylococcus aureus* pneumonia (123), depletion of TRPV1+ nociceptive neurons through *Trpv1-Dtr*, *Nav1.8-cre;Dta* or resiniferatoxin administration significantly improves survival and bacterial clearance, demonstrating nociceptor suppression of protective immunity. Mice lacking TRPV1+ neurons have improved neutrophil function and increased infiltration of lung-resident γδ T cells compared to vehicle control mice (123). Although a cough response was not directly assessed, these data demonstrate the extensive cross talk between airway neurons and host defenses against respiratory pathogens. Conversely, cough may manifest because the vagal nerves themselves can be directly subject to inflammation neuropathy. Influenza respiratory viral infection and exposure to the Gram-negative bacterial cell wall component lipopolysaccharide induce a state of vagal neuroinflammation, characterized by inflammatory cell recruitment, inflammatory gene induction and/or sensory neuron alarmin mobilization in the vagal sensory ganglia (124–126). Similarly, murine pneumovirus infections induce brainstem inflammation and alter synaptic efficacy in regions that process cough sensory inputs (127). Again, the functional consequences of pathogen-induced neuroinflammation with respect to cough have not been assessed, but similar processes in spinal sensory nerves underpin the development of somatic (e.g., pain) hypersensitivities.

TRANSMISSION OF INFECTIOUS PARTICLES

Infectious disease transmission commonly occurs through an airborne route, in which contact with infectious sources may be direct or indirect (8, 128). Direct contact occurs when aerosol droplets are inhaled by a susceptible host, whereas indirect exposure occurs through contact with contaminated surfaces. Aerosol droplets are emitted from the respiratory tract of an infectious individual and travel from the source to encounter the mucosa of a susceptible individual (8). Coughing and sneezing, two common symptoms of respiratory infection, are prototypical processes for generating aerosol particles (8). Understanding the role of cough-generated particles through human subject surveillance, animal transmission studies, and in silico modeling has increased preventative measures against airborne infectious disease transmission.

Successful transmission of Mtb depends on inhalation of airborne particles containing viable bacteria by a susceptible human host (129). However, many environmental and physiological factors can impact the infectiousness of those with active tuberculosis. Seminal work performed over 50 years ago using special cough monitoring equipment (130) demonstrated that nighttime cough was a frequent occurrence in pulmonary tuberculosis that was associated with severity of disease (131). Individuals with more severe disease on chest X-ray coughed more and were more likely to have close contacts that were tuberculin skin test positive (132). In addition, cough frequency, which averaged about 110 coughs over an 8-hour period on admission for active tuberculosis, declined rapidly after the onset of therapy (132). More recently, cough aerosol sampling of subjects with active tuberculosis shows those with high aerosol colony forming units (CFUs) are more likely to transmit infection to household contacts (129, 133). In addition to bacillary load, cough

frequency in active tuberculosis cases increases with lung cavitation during tuberculosis treatment, specifically when cavities are near the airway (134). Exacerbation of tuberculosis disease is associated with coinfections of HIV, comorbidities including diabetes, and drug-resistant Mtb strains (135). Analysis of cough dynamics through audio recording of active tuberculosis cases demonstrates increases in cough frequency in those with diabetes or recurrent Mtb infection and high CFU aerosols during drug-resistant Mtb infections (135, 136).

Sampling of cough aerosols from subjects with cystic fibrosis has also uncovered airborne transmission of pathogenic and opportunistic bacteria. Voluntary cough aerosols from cystic fibrosis subjects contain culturable *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *S. aureus*, demonstrating that airborne transmission of organisms that commonly cause infection for those with cystic fibrosis is feasible (137–139). Cough aerosols from subjects with non–cystic fibrosis bronchiectasis and chronic obstructive pulmonary disease also contain viable *P. aeruginosa*; however, genotypic analysis found no shared strains among study participants (140).

A major challenge in studying transmission of infectious diseases is the availability of animal models that effectively recapitulate human disease and transmission dynamics (141). Furthermore, transmission by cough-generated aerosols must also consider animal models that effectively cough and shed infectious agents. Airborne transmission by pertussis-infected baboons, an established model of whooping cough (87), occurs when naïve animals are cohoused with infected baboons or housed 7 feet away in a controlled environment (142). Airborne influenza A virus can be detected in cough-generated aerosols from human subjects (143), and small animal models demonstrate airborne transmission of influenza. However, it is unknown whether transmission is directly due to cough-generated aerosols or through indirect contact with infected animals (144–146). Ferrets are a commonly used small animal model for studying influenza pathogenesis, and similarly, they recapitulate disease symptoms of SARS-CoV-2 infection (147). Ferrets infected with SARS-CoV-2 experience elevated body temperature and occasional coughing compared to uninfected control animals. Furthermore, transmission of virus occurs through direct (cohoused) and indirect (permeable partition) contact between SARS-CoV-2-infected and naïve ferrets (147, 148).

In addition to human and animal studies, in silico modeling serves to further characterize cough-induced aerosol generation and transmission dynamics. As determined by mathematical modeling, among the 3,000 cough-generated respiratory droplets produced by a single cough, almost 400 can contain SARS-CoV-2 virus (149). Additionally, those with high viral load can expel 1.23×10^5 copies of virus from a single cough (149). Deposition of SARS-CoV-2 from cough-generated particles is highest in the extra thoracic airway based on the stochastic lung deposition model (150). Cough simulations have also been used to establish the efficacy of preventative measures against pathogen spread. Models of cough-induced droplet and aerosol spread reinforce the effectiveness (>90%) of facial coverings to reduce not only SARS-CoV-2 but also influenza exposure (9, 149). These data support the increased use of facial coverings and distancing as personal protection against SARS-CoV-2 infection.

CONCLUSIONS

Cough is a dynamic physiological process serving to clear the airways but can also be a sign of underlying pulmonary disease. Infectious diseases are uniquely poised to exploit the mechanical force generated by cough events to facilitate transmission of disease from person to person. Although experimental efforts are underway to characterize how infectious agents interact directly or indirectly with cough-inducing neurons, many unanswered questions remain in the field. A key question is, what are the evolutionarily conserved or distinct mechanisms across infections that

lead to cough? A wide variety of pathogens colonize the airway mucosa, and cough is a common and shared symptom across respiratory infection. Thus, one possibility is that pathogens of different origin may share conserved mechanisms to induce cough in infected individuals. Alternatively, each pathogen may have evolved unique approaches toward cough induction. To that end, are the host immune responses and accompanying secreted immune factors during pulmonary infection sufficient to initiate cough, or are molecules derived from infectious pathogens necessary to initiate or exacerbate the process? Related to this question, does cross talk between the airway immune microenvironment and airway neurons initiate or suppress cough? Finally, it is well established that cough-generated aerosol particles contain infectious agents and facilitate disease transmission. Thus, can the knowledge gained from studying the fundamental mechanisms of cough be leveraged to develop therapies to mitigate transmission via aerosol particles or droplets? In the context of ongoing and future pandemics of respiratory pathogens for which cough is a major vector of disease, further studies in infectious disease–related cough mechanisms and transmission dynamics are urgently needed.

SUMMARY POINTS

1. The airway epithelium is innervated by vagal sensory neurons, C-fibers, Aδ-fibers, and Aβ-fibers, which detect and respond to respiratory stimuli, including irritants, pathogens, and inflammatory mediators.
2. Cough-evoking stimuli interact with ligand-gated ionotropic receptors or metabotropic receptors to initiate a cough response.
3. Common respiratory pathogens encode toxins, lipids, and proteins that directly interact with cough-transducing receptors and activate cough.
4. Infection-induced inflammatory mediators act as agonists for cough-inducing receptors.
5. Sensory neurons regulate immune mechanisms and are subject to inflammatory neuropathy.
6. Cough-generated aerosol particles harbor infectious agents as a route of airborne transmission.

FUTURE ISSUES

1. Has cough evolved to protect the host from infection or been leveraged by airway pathogens to facilitate survival, improve replication, and/or enhance transmission?
2. Cough is a shared symptom among many respiratory infections. What are the conserved or distinct signaling mechanisms across infections that lead to cough?
3. How does the airway immune system impact infection-induced cough?
4. Can specific mechanisms of infectious cough effectively be targeted to mitigate airborne disease transmission?

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