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REVIEW

Current understanding of *Bordetella*-induced cough

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Abstract

Typical pathogenic bacteria of the genus *Bordetella* cause respiratory diseases, many of which are characterized by severe coughing in host animals. In human infections with these bacteria, such as whooping cough, coughing imposes a heavy burden on patients. The pathophysiology of this severe coughing had long been uncharacterized because convenient animal models that reproduce *Bordetella*-induced cough have not been available. However, rat and mouse models were recently shown as useful for understanding, at least partially, the causative factors and the mechanism of *Bordetella*-induced cough. Many types of coughs are induced under various physiological conditions, and the neurophysiological pathways of coughing are considered to vary among animal species, including humans. However, the neurophysiological mechanisms of the coughs in different animal species have not been entirely understood, and, accordingly, the current understanding of *Bordetella*-induced cough is still incomplete. Nevertheless, recent research findings may open the way for the development of prophylaxis and therapeutic measures against *Bordetella*-induced cough.

KEYWORDS

animal models, Bordetella, bradykinin, cough, deacylating autotransporter toxin, pertussis toxin, TRPV1

INTRODUCTION

Whooping cough, a respiratory infectious disease caused by *Bordetella pertussis* and occasionally *B. parapertussis*, has a long history with humans. The first record of this disease appeared in a treatise of the Sui Dynasty (518–608 ad) in China.[1] In this treatise (the Zhubing yuanhou lun), the disease thought to be whooping cough was categorized as a pediatric disease and referred to as "the cough of hundred days." The categorization as a pediatric disease is consistent with evident and severe symptoms of this disease in infants and children, and the designation "the cough of hundred days (百日咳)" is still used for whooping cough in China, Korea, and Japan. Besides whooping cough, other *Bordetella* species are also known to cause characteristic coughing in infected hosts. Thus, although *Bordetella*-induced cough has been widely recognized for a long time, its pathophysiological mechanism was totally unknown until recently. The primary obstacle to studying *Bordetella*-induced cough has been the lack of convenient animal models. However, recent studies utilizing rats and mice have moved the field forward by succeeding in quantifying cough numbers and providing insights into the coughing mechanism and causative factors of the bacteria.[2–5] This review aims to summarize the research history, the results of the latest studies, and the current knowledge of the pathogenesis of *Bordetella*-induced cough.

OVERVIEWS OF COUGH

Cough is a physiological response to protect the airways and lungs from inhaled or endogenous irritants. It can also be a sign of respiratory disorders arising from various diseases including microbial infections. However,

Abbreviations: AC, adenylate cyclase; ADR, adrenergic receptor; Bdk, bradykinin; B2R, bradykinin type 2 receptor; DAT, deacylating autotransporter toxin; GPCR, G-protein-coupled receptor; LOS, lipooligosaccharide; LPS, lipopolysaccharide; NA, noradrenaline; PKA, protein kinase A; PKC, protein kinase C; PTx, pertussis toxin; TRPA, Transient receptor potential ankyrin; TRPM, transient receptor potential melastatin; TLR4, toll-like receptor 4; TRPV, transient receptor potential vanilloid.

despite continuous research, the overall mechanism for coughing is not fully understood. Our current knowledge of cough, which is diverse but not comprehensive, is also arcane. Moreover, previous studies have underscored the variation in coughing mechanisms across different animal species and sexes in the presence or absence of anesthesia and with respect to the type of stimuli used. Therefore, in this review, to keep the focus on *Bordetella*-induced cough, the author introduces only basic knowledge of the general coughing mechanism and refers readers to other authoritative reviews for a detailed description of the physiology of cough.[6–9]

Neurophysiology of cough

The underlying physiological pathway to evoke cough can be summarized as follows. First, certain irritants or stimuli activate vagal sensory neurons (nociceptor neurons) mainly consisting of C-fibers and Aδ-fibers that innervate the airways and lungs. Subsequently, these vagal nerves transmit sensory information to the brainstem, where vagal afferent nerves synapse onto second-order neurons within the nucleus tractus solitarius. In this region, the reflex is processed, and the signal is then conveyed either through cholinergic parasympathetic efferents to induce reflex bronchoconstriction or via respiratory motoneurons to cause coughing. In addition, coughing is occasionally accompanied by an urge to cough, which involves cognitive processing by higher order brain functions. However, in view of the clinical manifestation of *Bordetella* infection in various host animals, *Bordetella*-induced cough is considered a reflex event.

Cellular factors involved in evoking cough[7–11]

Ion channels expressed on sensory nerves and surrounding tissues are considered to generate an action potential on sensory neurons to cause the cough reflex. Of these, transient receptor potential (TRP) channels, which conduct cations in response to extracellular stimuli, including temperatures, chemicals, stretch, pH, osmolarity, and others, have been the primary focus of studies in relation to cough events. TRP vanilloid 1 and 4 (TRPV1 and TRPV4), ankyrin 1 (TRPA1), and melastatin 8 (TRPM8) are reportedly involved in sensory nerve activation leading to cough. Trimeric P2X3 receptors consisting of three P2X3 subunits or two P2X3 and one P2X2 subunit are members of the purinergic P2X family of ion channels, which are activated by ATP or its derivatives. Upon activation, these ion channels depolarize airway sensory nerves to evoke cough. In addition, previous studies pointed out that voltage-gated sodium channels play roles as nociceptors within the respiratory tract and generate action potentials for evoking cough.

Physiologically active substances, both endogenous and exogenous, are known to modulate or amplify the action of the above-mentioned channels. The endogenous substances include prostanoids (e.g., prostaglandins, leukotrienes, and thromboxanes), activated amines (e.g., histamine, serotonin, and noradrenalin), tachykinins (i.e., neurokinins and substance P), and bioactive peptides (e.g., bradykinin (Bdk) and calcitonin gene-related peptide); some of which are released from activated sensory nerve cells or induced by inflammatory responses in hosts. These substances function via their respective specific receptors, many of which are G-protein-coupled receptors (GPCRs) (e.g., receptors for prostaglandins, Bdk, histamine, and catecholamine). In such cases, the action of these factors is determined by the type of GTP-binding protein that is coupled with GPCRs and the type of ion channels targeted by the GTP-binding protein.

Animal models for cough research

To develop antitussives targeting the core system evoking cough, efforts to understand the neurophysiology of cough have long been made using animal models. Dogs, cats, rabbits, guinea pigs, rats, and mice have been utilized under anesthesia or nonanesthesia.[6] The animals are provoked to cough with mechanical or chemical stimuli, such as scratching with a catheter, citric acid, capsaicin, or inflammatory modulators. Coughing is defined and evaluated by pleural pressure, electromyograms of the diaphragm or abdominal muscles, postures of coughing, airflow waveforms recorded by plethysmography, coughing sounds, and other methods. The use of larger animals, including dogs, cats, and rabbits, was recently restricted because of ethical and cost issues. Guinea pigs are the most frequently utilized animal model to analyze coughing. The coughing response of guinea pigs to chemical stimuli is reportedly similar to that of humans.[12] In vitro analyses also indicated that the vagal nerve of guinea pigs depolarized in a similar manner to the human vagal nerve.[6,13] The European Respiratory Society has recommended conscious guinea pigs as the most suitable coughing model,[6,13,14] and guinea pig models have provided extensive information on the physiology and pharmacology of the cough reflex. Rats and mice, which are widely used in life sciences, have long been considered improper as animal models for coughing. Some literature pointed out that the main reflexogenic area of rats is the larynx rather than the trachea and bronchi and that the cough-like response of rats must be an expiration reflex and not a cough. Similarly, there are opposing views regarding the use of mice in cough experiments because past studies failed to observe epithelial nerves in the lower airways of mice.[6,15,16] Some researchers claimed that mice are too tiny to generate a strong pressure gradient across the respiratory tracts to produce cough expulsion, and any ventilation changes leading to cough are weak and difficult to record.[6,13] However, an increasing number of reports of mouse coughing have appeared in recent years.[17–23] Moreover, *Bordetella*-induced cough has been analyzed with rat and mouse models, as mentioned below.

BORDETELLA-INDUCED COUGH

Bordetella species that cause cough in infected hosts

Currently, 16 named *Bordetella* species are recognized.[24,25] Of these, *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*, which are correctively referred to as the classical *Bordetella* species, commonly cause respiratory infections with severe coughing episodes in humans and other mammals. Human infections by *B. pertussis* and *B. parapertussis* are known as whooping cough and whooping cough-like disease, respectively. Occasionally, *B. parapertussis* infection is also called whooping cough because it is clinically indistinguishable from *B. pertussis* infection. The disease manifestation of *B. bronchiseptica* infection is milder than that of *B. pertussis* and *B. parapertussis*; however, pigs, dogs, rabbits, and rarely humans infected with *B. bronchiseptica* are known to exhibit coughing.[2,26–31] The classical *Bordetella* species share a variety of homologous virulence factors, implying that the bacteria cause coughing through a similar mechanism that involves these factors. However, as described below, recent works have revealed that the mechanism of paroxysmal coughing caused by the classical *Bordetella* species is more complex than initially anticipated.

Besides the classical *Bordetella* species, *B. holmesii* is often isolated from patients with whooping cough-like illness. Since this organism was first documented in 1995,[32] some reports have claimed that *B. holmesii* is a causative agent for coughing clinically similar to that of whooping cough; however, this argument is yet to be verified because the involvement of other microorganisms in a potential co-infection cannot be ruled out. In addition, there is no experimental evidence that *B. holmesii* directly causes coughing in animals.[27,33,34] Other *Bordetella* species infect birds or rodents and are occasionally isolated from humans or environmental sources. No information about the ability of these *Bordetella* species to produce cough is available.

B. pertussis-induced cough

Animal models

Nonhuman primates have long been tested as promising experimental animals to reproduce pertussis cough. Previous studies in the 1920s to 1930s reported that macaques developed paroxysmal coughs after inoculation of the bacteria; however, some studies failed to establish *B. pertussis* infection.[35–42] More recent work also reported that infant rhesus macaques showed severe coughing by exposure to bacterial aerosol or transmission from infected animals.[43] Another research group indicated that baboon was preferable to reproduce pertussis cough than rhesus macaque, which responded differently to *B. pertussis* infection in different individuals, and that all baboons inoculated with the bacteria developed severe coughs that persisted for over 2 weeks.[44] However, using macaques and baboons for analyses of cough mechanisms, which require a large number of individuals, is less feasible for animal welfare and cost reasons.

Coughing (or sneezing) of rats inoculated with *B. pertussis* was first reported in 1939.[45] In that study, the cough appeared on average 8.3 days postinoculation in 12 rats and lasted for 60 days in some cases. However, the frequency of the cough was not reported. Fifty years later, coughing in rats due to *B. pertussis* infection was reproduced with a modified inoculation method, in which bacteria encased in agarose beads were introduced into the bronchus of tracheotomized rats[46]; however, the details of coughing were not described. Thereafter, this inoculation method was adopted by another research group, and coughs were recorded by a voice-activated tape recorder.[47–51] They defined repetitive chirping or coughing sounds recorded on the tape as paroxysms and counted the number of paroxysms each night for 21 days. Several of those studies reported that the coughing started around 5 days postinoculation, and its frequency reached a maximum from Days 8 to 14. Heat-killed *B. pertussis* did not cause cough paroxysms. Pertussis toxin (PTx), but not dermonecrotic toxin (heat-labile toxin), was necessary for the cough production. *B. parapertussis* did not cause coughing in this model. Vaccination with diphtheria–tetanus–pertussis (whole-cell) vaccine protected rats from cough paroxysms. The extent of the cough responses differed among different rat strains: Sprague–Dawley rats were more susceptible than Brown Norway, Lewis, and Hooded Lister rats in the cough response. However, because the studies did not evaluate the level of bacterial colonization for each experiment, it is difficult to know whether the inability of bacteria to produce coughing was due to the inability to trigger cough responses or the inability to efficiently colonize. Although the inoculation procedures in this model were cumbersome and no research groups followed up on these studies, this coughing rat model pioneered an approach to explore the pathogenesis of pertussis cough and has provided fundamental information. Coughing in rats inoculated with naked *B. pertussis* was again confirmed in 2019.[2] These experiments were carried out to compare coughs caused by *B. bronchiseptica*. The degree of *B. pertussis*-induced cough in rats varied widely among individuals compared to that of *B. bronchiseptica*-induced cough, and therefore, this study concluded that the rat model is unsuitable for analyses of *B. pertussis*-induced cough.

The newborn piglet was also reported to exhibit respiratory symptoms including nonparoxysmal cough after the intrapulmonary inoculation of *B. pertussis* encased by agar beads; however, details of the coughing were not described.[52] Mice have been the most widely used animal model for analyzing the pathogenesis, immune responses, and vaccine efficacy in relation to *B. pertussis* infection. However, as mentioned above, it has long been believed that mice cannot cough,[38,53] and, therefore, this animal had not been utilized for cough analyses until 2022.[5]

Mechanism of *B. pertussis*-induced cough[5]

The study of the mechanism of pertussis cough was significantly advanced by the use of mice, which provide various genetically modified mutants. This study revealed that C57BL/6 mice cough after *B. pertussis* infection, but BALB/c mice do not. Coughs of C57BL/6 mice confirmed by whole-body plethysmograms were observed 8–10 days after intranasal inoculation of the bacteria and persisted for at least 7 days. Intranasal inoculation of the bacterial lysate as well as the living bacteria also caused coughing; this fact facilitated the analysis of the cough-inducing mechanism because the extent of bacterial colonization could be excluded as a factor affecting the magnitude of the cough production. As a result, the study revealed that the lipid A moiety of lipooligosaccharide (LOS), autotransporter Vag8, and PTx of *B. pertussis* cooperatively cause coughs by hypersensitizing TRPV1, which resides on sensory nerve terminals and generates action potentials to evoke cough reflex. The clarified mechanism is as follows (Figure 1): LOS stimulates the production of Bdk, an inflammatory mediator, by interacting with lipid A-specific toll-like receptor 4 (TLR4). Autotransporter Vag8 further enhances Bdk production by inhibiting C1 esterase inhibitor,[55–59] which negatively regulates the kallikrein–kinin system in the plasma contact system producing Bdk. Accumulated Bdk interacts with Bdk type 2 receptor (B2R), which is coupled with the G_q and G_i families of heterotrimeric GTPases. G_q and G_i transduce stimulatory and inhibitory signals down to TRPV1, respectively. PTx ADP ribosylates the G_i α subunit ($G\alpha_i$)[60] and uncouples the B2R–G_i pathway but not the B2R–G_q pathway. Consequently, TRPV1 is hypersensitized via the B2R-G_q stimulatory pathway and readily causes nervous excitation to evoke cough by innocuous stimuli. In this study,[5] several genetically modified mice contributed to dissecting the cough mechanism, including TLR4-knockout (*Tlr4*$^{-/-}$) mice, high-molecular-weight kininogen-knockout (*Kng1*$^{-/-}$) mice, which have a defect in Bdk generation, and

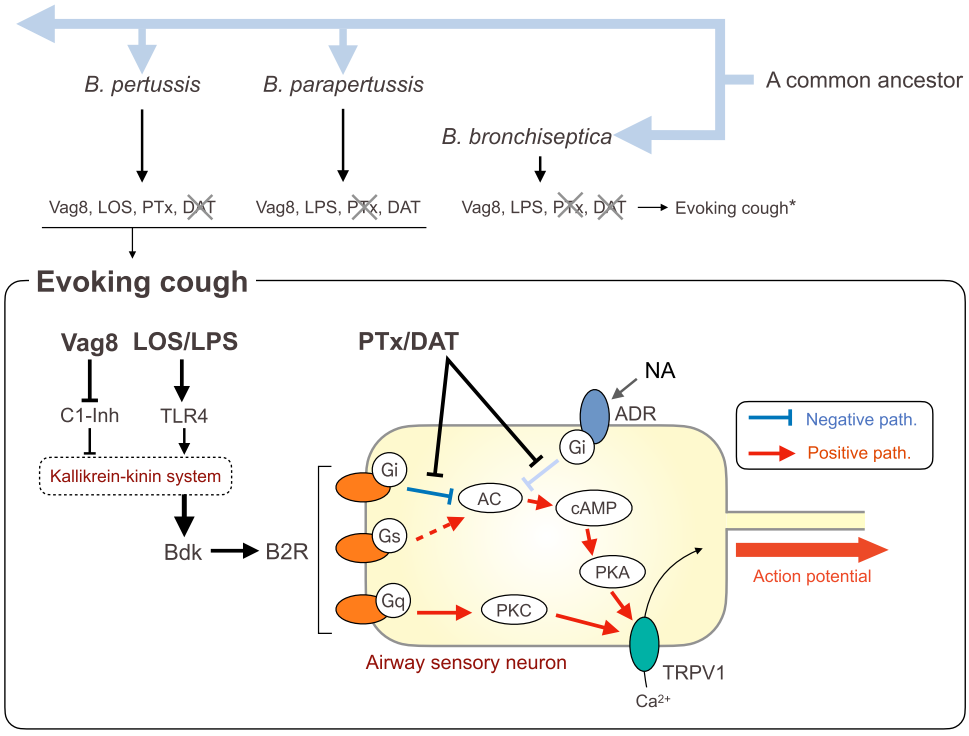

FIGURE 1 Mechanism of cough induced by *Bordetella pertussis* and *B. parapertussis*. Upper panel: Comparative genomics revealed that *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* independently evolved from a common ancestor, to which *B. bronchiseptica* is closely related.[29,54] *B. pertussis* and *B. parapertussis* evoke coughing through the action of Vag8, lipooligosaccharide/lipopolysaccharide (LOS/LPS), and pertussis toxin/deacylating autotransporter toxin (PTx/DAT) as shown in the lower panel. **B. bronchiseptica*, which produces Vag8 and LPS, but neither PTx nor DAT, also evokes coughs in some host animals; however, its mechanism is apparently different from that of *B. pertussis* and *B. parapertussis*. Lower panel: The mechanism of coughing induced by *B. pertussis* and *B. parapertussis*. LOS of *B. pertussis* and LPS of *B. parapertussis* stimulate bradykinin (Bdk) generation by the kallikrein–kinin system through interaction with toll-like receptor 4. Vag8 inhibits C1 esterase inhibitor (C1-inh), which negatively regulates the kallikrein–kinin system, and accelerates Bdk generation. The accumulated Bdk transduces stimulatory signals via the B2R–G_q–PKC pathway to transient receptor potential vanilloid 1 (TRPV1), which generates action potential to evoke the cough reflex. Simultaneously, B2R transduces an inhibitory signal via the B2R–G_i–AC–cAMP–PKA pathway. Although Bdk also transduces a stimulatory signal via B2R–G_s, the G_i pathway works dominantly over the G_s pathway. PTx of *B. pertussis* and DAT of *B. parapertussis* inhibit only the B2R–G_i–AC pathway, and therefore, TRPV1 is hypersensitized by the G_q and G_s pathways. Consequently, TRPV1 comes to respond even to innocuous stimuli to evoke coughs readily. Besides, PTx and DAT probably inhibit the presumed negative regulation systems for the cough reflex that are transduced by G_i-coupled receptors, such as NA-stimulated α2-adrenergic receptors; however, these negative regulation systems for the cough reflex are predicted but remain to be identified. AC, adenylate cyclase; ADR, adrenergic receptor; B2R, Bdk type 2 receptor; NA, noradrenaline; PKA, protein kinase A; PKC, protein kinase C.

TRPV1-knockout mice, all of which exhibited less frequent coughs induced by the bacteria than wild-type mice, confirming that the TLR4–Bdk–TRPV1 pathway evokes cough.

B. parapertussis-induced cough

Pathway to evoke coughs by *B. parapertussis*

B. parapertussis-induced cough was first examined in a rat model study that mainly analyzed *B. pertussis*-induced cough.[49] In this study, *B. parapertussis* did not cause cough, and coughing by *B. parapertussis* has long been unstudied. Recently, *B. parapertussis* cough was observed in a mouse model similar to that through which *B. pertussis*-induced cough was analyzed[4]: C57BL/6 mice were found to cough approximately 6 days after the intranasal inoculation of *B. parapertussis*, and the cough persisted for at least 7 days, providing new insights into the cough mechanism.[4] The mice also exhibited cough after inoculation with lysates of *B. parapertussis*, similar to *B. pertussis*. Like *B. pertussis*-induced cough, the frequencies of *B. parapertussis*-induced cough were significantly reduced in *Tlr4*$^{-/-}$ mice and *Kng1*$^{-/-}$ mice. Antagonists against B2R and TRPV1 also lowered the frequency of coughs induced by *B. parapertussis*. These results indicated that *B. parapertussis* evokes coughing in mice through the same pathway as *B. pertussis* (Figure 1). Indeed, *B. parapertussis* produces lipid A as a part of lipopolysaccharide (LPS) and Vag8, which is 97.3% identical to Vag8 of *B. pertussis*. However, *B. parapertussis* does not produce PTx due to nucleotide alterations that inactivate the promotor of the operon and make *ptxB* a pseudogene.[54,61] Instead, a novel toxin of *B. parapertussis*, deacylating autotransporter toxin (DAT), shuts down the G_i-dependent signal pathway as follows.

DAT

DAT belongs to the family of autotransporter proteins, which consist of passenger and translocator domains. The passenger domain of DAT contains a GDSL lipolytic enzyme motif and dissociates the myristoyl group attached to $G\alpha_i$, resulting in the interruption of $G\alpha_i$-dependent signaling because the myristoyl group on $G\alpha_i$ is indispensable for the interaction with adenylate cyclases, the downstream effectors. Thus, DAT exacerbates the Bdk-induced hypersensitization of TRPV1 in *B. parapertussis* infection in place of PTx in *B. pertussis* infection. Indeed, intranasal inoculation of the combination of LPS, Vag8, and DAT in *B. parapertussis* caused coughing in mice, similar to the combination of LOS, Vag8, and PTx in *B. pertussis*. *B. pertussis* does not produce DAT since it lacks the *dat* gene.

B. bronchiseptica-induced cough

Attempts to understand the pathogenesis of coughing induced by *B. bronchiseptica* were first reported in 2019.[2] This study, which utilized intranasally inoculated rats with naked (not embedded in agarose beads) bacteria or bacterial lysates, demonstrated that a heat-labile and Bvg$^+$ phase-specific factor, but not adenylate cyclase toxin, dermonecrotic toxin, or type III secretion effectors, is involved in the cough production. *B. bronchiseptica* does not produce PTx because the *ptx* promoter is inactivated by nucleotide substitutions.[54,61,62] This study also isolated a spontaneous mutant strain with a reduced ability to cause coughing. The genome sequencing of the mutant strain revealed a single base deletion in the *btrA* gene (also called *bspR*) encoding an anti-σ factor, and the following rat coughing experiments with a *btrA*-deficient mutant indicated that BtrA regulates the ability of the bacteria to cause cough. BtrA negatively or positively regulates sets of downstream virulence genes, antagonizing BtrS, an extracytoplasmic function σ factor.[63,64] In addition, BtrA is secreted by the type III secretion system and translocated into the nucleus of target cells, suggesting its function as a type III-secreted effector but whose molecular action remains unknown.[65] Because a mutant of *B. bronchiseptica* deficient in type III secretion caused coughing, secreted BtrA is unlikely to be involved in cough production. Rather, the contribution of BtrA to cough production as a regulator of gene expression is more probable. Collectively, the cough factor of *B. bronchiseptica* is heat labile and expressed specifically in the Bvg$^+$ bacterial phase under the regulation of BtrA. *B. bronchiseptica* has an intact *dat* gene but does not produce DAT for unknown reasons.[4] Lysate of Vag8-deficient *B. bronchiseptica* induced cough in mice to the same extent as lysate of the wild-type strain, and substances that amplify Bdk-dependent increases in cAMP by inhibiting $G\alpha_i$-dependent signaling, such as PTx and DAT, were not found in *B. bronchiseptica* lysates (unpublished data). These results suggest that *B. bronchiseptica* induces cough in host animals through a pathway distinct from Bdk–TRPV1.

B. holmesii

As mentioned above, whether *B. holmesii* causes host coughing is yet to be confirmed. Because *B. holmesii* does not carry orthologue genes for Vag8 and DAT, the mechanism of *B. holmesii*-induced cough, if any, must be totally different from *B. pertussis*- and *B. parapertussis*-induced coughs. An appropriate animal experiment system to dissect the cough mechanism by *B. holmesii* has yet to be established. Furthermore, there is no available information as to whether mice cough after *B. holmesii* inoculation.

REMAINING ISSUES AND PERSPECTIVES

The mouse cough model has become a powerful tool in elucidating the mechanisms of *Bordetella*-induced cough. Although there is still controversy as to whether mice cough or not, the fact that experimental infection with *B. pertussis* or *B. parapertussis*, which causes severe coughing in humans, has caused respiratory reflexes in mice cannot be overlooked by *Bordetella* researchers. As reports regarding mouse coughing are currently accumulating, it is hoped that experts in the physiology of cough will re-examine the nature of it. The mouse model of *Bordetella*-induced cough, which exhibits frequent coughs without any stimuli, may be helpful for the study.

It has been clarified that the TLR4–Bdk–B2R–TRPV1 pathway mediates coughing evoked by *B. pertussis* and *B. parapertussis* infection as mentioned above; however, *Tlr4*$^{-/-}$, *Kng1*$^{-/-}$, and TRPV1-knockout mice still cough, although less frequently, in response to intranasal inoculation with bacterial lysates or LOS–Vag8–PTx, suggesting that other mediators and ion channels are involved in evoking cough in addition to Bdk and TRPV1.[4,5] In addition, the ability to cause coughing varies a great deal across *B. pertussis* strains: for example, a representative laboratory strain of *B. pertussis*, Tohama, hardly causes coughing in mice even though it produces LOS, Vag8, and PTx at the same level as other strains.[5] Since the combination of LOS, Vag8, and PTx from the Tohama strain caused mouse coughing, an uncovered mechanism that modulates the cough-inducing ability of the bacteria may be present. Thus, further work is required to understand the entire mechanism of *Bordetella*-induced cough and develop prophylactic and therapeutic agents for human cases.

Genome analyses of classical *Bordetella* spp. revealed that *B. pertussis* and *B. parapertussis* independently evolved from an ancestor that is most closely related to *B. bronchiseptica* (Figure 1).[29,54] *B. pertussis*, which does not produce DAT, utilizes PTx to cause cough. *B. parapertussis*, which does not produce PTx, utilizes DAT to cause cough. In addition, our unpublished data suggest that *B. bronchiseptica*, which produces neither PTx nor DAT, likely causes cough through a mechanism apparently different from *B. pertussis* and *B. parapertussis*. This observation implies that classical *Bordetella* may need to induce severe cough in host animals, even if by different means, to maintain their life cycle or infectivity. Thus, further understanding of the cough-inducing mechanism by *Bordetella* spp. may provide insights into the biology of *Bordetella*.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data described in this article can be referred to the corresponding references.

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