Trends in Microbiology

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Review

Integrated Signaling Pathways Mediate *Bordetella* Immunomodulation, Persistence, and Transmission

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The mammalian immune system includes a sophisticated array of antimicrobial mechanisms. However, successful pathogens have developed subversive strategies to detect, modulate, and/or evade immune control and clearance. Independent disciplines study host immunology and bacterial pathogenesis, but interkingdom signaling between bacteria and host during natural infection remains poorly understood. An efficient natural host infection system has revealed complex communication between *Bordetella* spp. and mice, identified novel regulatory mechanisms, and demonstrated that bordetellae can respond to microenvironment and inflammatory status cues. Understanding these bacterial signaling pathways and their complex network that allows precisely timed expression of numerous immunomodulatory factors will serve as a paradigm for other organisms lacking such a powerful experimental infection system.
Video abstract

Highlights

Well adapted bacterial pathogens have evolved mechanisms to sense and respond to immune components and host molecules, enabling them to evade, manipulate, or escape their antimicrobial effects.

Bacteria adapt to particular anatomical sites and inflammatory stages by sensing signals specific to each and changing gene expression accordingly.

Two-component systems, sigma factors, sRNAs, and chaperones allow bacteria to respond to environmental changes by orchestrating gene expression to optimize their fitness.

Sensing carbon dioxide, iron starvation, hormones, and likely many other signals can refine the precise expression of virulence factors.

Optimal temporal and spatial expression of virulence factors allows effective manipulation of host immune responses, and allows bacteria to efficiently colonize, infect, persist, and increase transmission amongst hosts.

Bacterial Sensing of the Environment

The ability of all bacteria to sense and respond to environmental cues is an important feature for their evolutionary success [1–6]. Gram-negative bacteria can increase growth in the presence of catecholamines, suggesting a path for bacteria to respond to host hormones [7]. Sperandio *et al*. enhanced our knowledge of cross-kingdom communication, showing that bacteria are able to respond to host molecules such as hormones by increasing the expression of virulence factors [4–6]. In recent years there have been significant advances in the understanding of host immune signaling, establishing that host signals at different postinfection times trigger bacterial responses that can impact the host [8–11]. But, despite the numerous developments in both fields, and accumulating evidence that bacteria sense the host and adapt gene expression accordingly, most work in the field has been done separately in the host, or bacteria, or *in vitro*. When *in vivo* experiments are performed to study both signals in conjunction, most of the work is using bacteria that are well studied in regard to quorum sensing but are not natural pathogens of the animal models, leading to an immune response that is of questionable relevance to that of natural infection. These models lack the well-evolved relationship of host and pathogen, and lack key host responses (such as different glycosylation patterns) and bacterial strategies (such as intracellular survival) to overcome them, giving an incomplete picture of the natural host–pathogen interactions.

Bordetella spp. include commensals (such as *B. bronchiseptica*) and serious pathogens (*B. pertussis* or *B. parapertussis*). The dynamics of the host–pathogen relationship is largely determined by a variety of signals exchanged between the two organisms, each sensing aspects of the other. For example, the host can detect bacterial components, secreted

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effectors, and/or (bi)products of bacterial metabolism. Bacteria, in turn, can sense the many host antimicrobial defenses, and can even intercept intracellular signaling molecules [4,12]. The great complexity of all of these potential interkingdom communications and our poor understanding of them make it impossible to simulate most aspects of these interactions, leaving us dependent on animal infection systems for their study. Unfortunately, there are few such experimental systems that can be used to study the intricate, complex, and well-evolved interactions where both bacterial and host can be manipulated at the molecular level. *Bordetella* spp. include natural and highly efficient pathogens and commensals of mice, providing an exceptional experimental setting to study the communication between bacteria and the host immune response. Bordetellae are able to respond to environmental changes, including those of the environment as well as those expected to vary in different host microenvironments and in response to infection, sensing hormones, iron, or CO_2 levels, for example [13–16]. The pathways that regulate these behavioral changes, however, remain unclear. Thus far, BvgAS has been described as the master regulon of *Bordetella* spp. virulence factors, but with the discovery of other two-component systems (TCSs) as well as sigma factors, small RNAs (sRNAs) and chaperones, there is increasing evidence that these sensor-response systems are more complicated than previously thought [17–25].

In this review we examine the regulatory mechanisms that have been reported in *Bordetella* spp. We describe how bordetellae can sense different environments within the host and express virulence factors that enable them to manipulate host immunity, cause persistent/chronic infection, and ultimately facilitate transmission. Finally, we postulate a perspective that will provide new insight into the ability of *Bordetella* spp. to sense host molecules to trigger a response that will lead to modulation of inflammation and ultimately enable persistence within the host.

Bordetella Responds to the Host Environment

Mammalian hosts have a long list of antimicrobial effectors, so bacteria are under strong selective pressure to evolve methods to sense and respond to evade them. While these mechanisms to sense and respond to environmental cues have been studied in several organisms, such as *Vibrio* and *Pseudomonas* spp. [26–33], *Bordetella* spp. have particularly powerful experimental systems that allow them to be studied during natural host infection *in vivo*.

There is growing evidence that bacteria and host communicate via diffusible molecules that can be recognized by bacteria, altering gene expression [1,4–6,31–33]. One of the most universal mechanisms for resisting bacterial infection is iron sequestration. The level of available iron in the host is a limiting factor for bacterial growth, and, consequentially, bacteria have evolved different mechanisms of iron acquisition, including the use of siderophores such as enterobactin, allowing them to divert iron from the host. Armstrong *et al*. hypothesized that *Bordetella* spp. may be exposed to nutritionally relevant catecholamine concentrations in the local microenvironment during infection, either on the mucosal surface via serum exudation or through interaction with immune cells [34]. Noradrenaline has been shown to have a strong iron-shuttling activity, allowing *Bordetella* spp. to use catecholamines as shuttles that enhance ferric iron uptake from any source available. In the absence of siderophores, catecholamines can remove the iron from transferrin and lactoferrin to directly bind it to *B. bronchiseptica* [13]. The mechanisms by which these neuroendocrine catecholamines – including epinephrine, norepinephrine, and dopamine – function is by increasing transcription of *bfeA* (outer membrane protein) of *B. bronchiseptica*, which is involved in transporting ferric catechol [13,34].

The primary function of the respiratory tract, gas exchange, leads to a steep increase in CO_2 concentrations across the small distance from the air–liquid interface to the cell–liquid interface. The ability to detect this range of CO_2 concentration may allow bacteria to sense their relative position within airway fluids and respond with appropriate gene expression patterns. Consistent with these predictions, bordetellae exposed to CO_2 levels in this range (ambient air to 5% CO_2) alter their expression of many genes implicated in infection [15]. In 5% CO_2, *B. bronchiseptica, B. pertussis*, and *B. parapertussis* alter the expression of adenylate cyclase toxin (ACT), secretion systems, pertactin (PRN), and other virulence factors [15].

During disease progression, the inflammatory response is active, which means that blood components will reach the respiratory tissue to deliver immune cells and other antimicrobials. All the classical *Bordetella* species – *Bordetella bronchiseptica, B. pertussis*, and *B. parapertussis* – can sense blood and serum components and respond with altered expression of various antigens and increased expression of various known virulence factors and iron-scavenging systems [35]. They also change phenotypically, increasing their cytotoxicity likely due to the increased expression of virulence factors such as ACT and/or the type 3 secretion system (T3SS) [35]. Gonyar *et al*. studied those host factors that increase the expression of a particular toxin and found that exposure of *B. pertussis* to fetal bovine serum, albumin, and calcium increases ACT levels, suggesting that *B. pertussis* can sense the presence of those two specific serum components and respond by increasing production of ACT – which not only works as a hemolysin but also plays a major role in the downregulation of the host inflammatory response [16].

In response to the previously mentioned signals – catecholamines, iron, CO_2, blood, and serum – *Bordetella* spp. respond by increasing expression of sets of genes known as virulence factors that include ACT, pertussis toxin (PTX), T3SS, and filamentous hemagglutinin (FHA), most of which are known for their ability to manipulate the inflammatory and/or immune response. Recent work reveals intriguing hints, admittedly only partially understood, of how these virulence factors may manipulate host responses. But current knowledge and theory about the dynamics of bacteria–host interactions predict that, for optimal effect, each virulence factor must be carefully regulated and expressed precisely in the appropriate conditions to have their optimal effect in a narrow window before adaptive immunity (e.g., antibodies) inhibits their effects. Therefore, *Bordetella* spp. must be able to detect cues of different host locations and conditions, such as inflammation, and alter gene expression to respond to the challenges and opportunities for immunomodulation in each. This argument leads to important questions: how can *Bordetella* spp. sense the environmental changes, and what are the underlying mechanisms that regulate their responses? Can we manipulate these mechanisms/pathways to design new vaccines and/or therapies? The answers could reveal new targets and/or tools to develop therapies and vaccines.

Molecular Mechanisms of Sensing

Bordetella spp. harbor multiple mechanisms to sense and respond to environmental cues, including TCSs, sigma factors, sRNAs, and chaperones, amongst others. Abundant research has been effective in unraveling the mechanisms and molecular connections between different signaling pathways in several organisms, including *Bordetella* spp., *Pseudomonas aeruginosa, Escherichia coli*, and *Salmonella* spp. Most of the studies, however, have been done *in vitro*. Here we review the molecular pathways that *Bordetella* spp. utilize to sense and respond to stimuli in the context of the powerful experimental infection system in which immunomodulation can be studied during natural host infections.

TCSs

Three TCSs have been implicated in the interactions between *Bordetella* spp. and their hosts: *BvgAS*, *RisAS*, and *PlrSR* (Figure 1; see also supplemental information online). TCSs depend on a kinase, activated by conformational changes [36], to propagate signals that ultimately affect gene regulation [37]. Kinases are activated by phosphorylation in response to external signaling. This signaling can be a consequence of any environmental change or imbalance to which the cell is sensitive [17,20,38]. Once active, the kinase will vary the levels of phosphorylated BvgA, RisA, or PlrR [18–20,38–41]. The changes in gene transcription propagated from the TCSs are necessary for *Bordetella* spp. virulence [22,42], colonization, persistence, and ultimately transmission, but more studies need to be done to better understand the specific roles of these in adaptation and response to different host molecules, including those specific to the response to host surveillance.

BvgAS TCS

The prototypical master regulator of virulence in *Bordetella* spp. is the Bvg (*Bordetella* virulence gene) system [22,25,43]. The expression of genes regulated by Bvg varies based on the environmental circumstances. This creates a phase system in which the bacterium transitions between states of differential gene expression. Although a continuum exists between them, there are three recognized and described phases of this system: plus (Bvg^+), minus (Bvg^-), and intermediate (Bvg^i) [18,43]. The Bvg^+ phase is often thought of as the virulent state and is required for infection of the mammalian host, while the Bvg^- phase

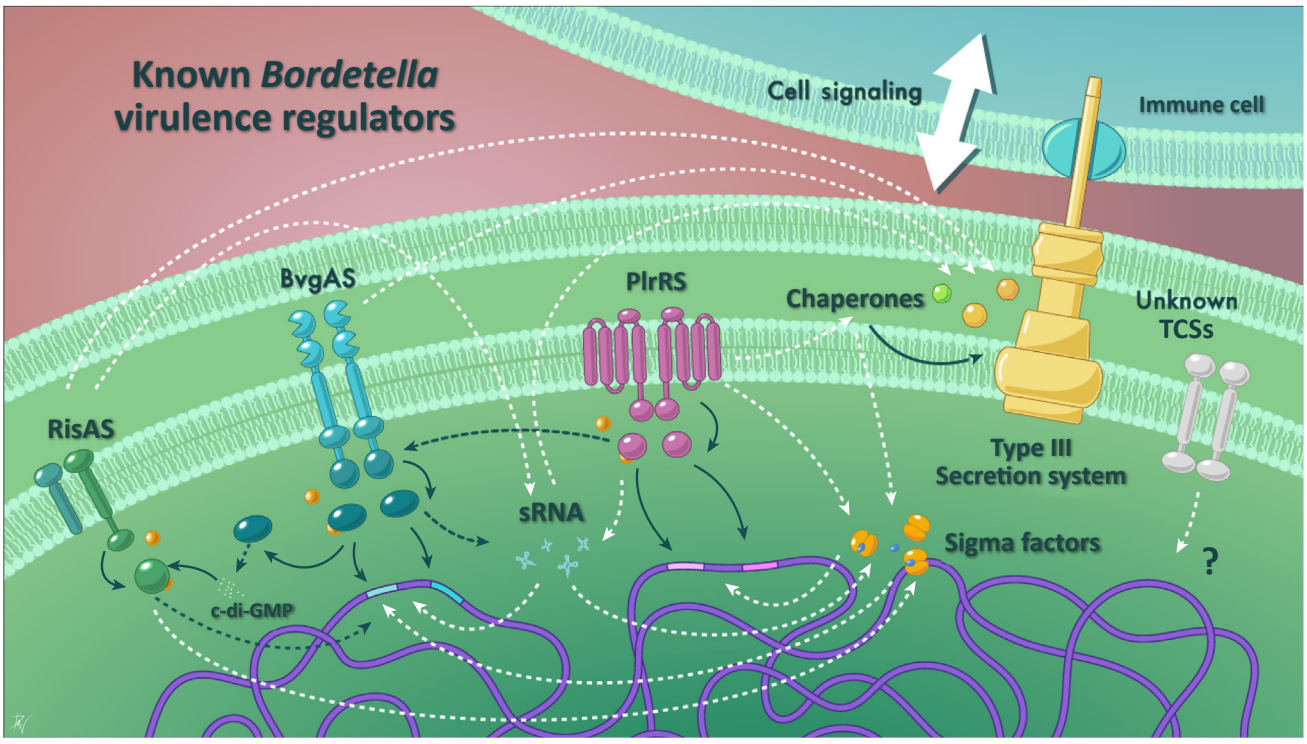


Figure 1. Model of the Molecular Pathways of Sensing and Responding in *Bordetella* spp. – A Schematic Representation of the Hypothetical Connections between the Regulatory Mechanisms of Different *Bordetella* spp. The dotted lines indicate networks that are hypothetical. The bidirectional arrow indicates that the regulation can hypothetically go either way. The question mark indicates that there is no knowledge about the function of those regulators.

is necessary for survival outside the mammalian host and mediates newly discovered interactions with amoebae [43]. The Bvg^+ phase is needed for infecting the mammalian host, where nutrients are abundant and the temperature is favorable (about 37° Celsius) [43]. The Bvg^- phase is an avirulent phase characterized by an increase in motility. It occurs at room temperature and is important to *in vitro* assays. The Bvg^- phase can be induced in laboratory conditions by addition of magnesium sulfate in the medium [43]. Specifically, under these conditions, *vrg* membrane proteins are highly expressed [44]. The transition from the Bvg^+ phase to the Bvg^- phase can occur intracellularly but appears to be species dependent [44]. FHA is still expressed while toxin production is diminished [43]. Biofilms can form during this period as a result of high FHA expression. Formation of a biofilm makes treatment difficult and increases chronicity [43]. Thus, genes regulated by the Bvg system are related to motility and virulence [45].

In the *Bordetella* field there is a paradigm in which Bvg^+ is the virulent phase associated with the host, and Bvg^- is the environmental phase, but this is likely an oversimplification. Although it is well established that Bvg^+ mutants can colonize mice while Bvg^- phase-locked mutants do not [46], recent literature has shown that things are not this simple, and other TCSs can differentially be involved in the regulation of the Bvg system [20,38] to work jointly in the adaptation of *Bordetella* spp. to different environments.

There are still important questions that remain, such as what is the biological function of the Bvg intermediate phase? Or does Bvg control other regulators such as sigma factors, chaperones, and sRNAs? Importantly, some genes that are optimally expressed in the Bvg^- phase are necessary for the infective cycle of the pathogens. For example, the transmission-associated exopolysaccharide (tEPS) locus is induced in the Bvg^- phase and has only a modest effect on colonization in the murine model, but mutants lacking the tEPS are deficient in transmission [47]. These findings, and the observation that *B. pertussis* and *B. parapertussis* retain parts of the Bvg^- phase, indicate that it retains some function in their closed life cycle in their human host.

The respiratory system contains a highly variable environment, with gradients of temperature, O_2 and CO_2, nutrients, and a constellation of host molecules that are accessible in variable concentration. Although the Bvg system was once thought to be a simple switch that turned on/off all the virulence factors, it is now recognized that many signals of the microenvironment affect gene expression in complex ways, some of which involve interactions with Bvg and crosstalk between signaling pathways.

RisAS TCS

RisAS (reduced intracellular survival) interacts with, and can act in some ways in opposition to, Bvg [40,48]. While it may interact with Bvg, RisAS does not require *Bvg* for proper expression [41]. The RisAS system is orthologous to the EnvZ/OmpR system present in other Gram-negative bacteria, and it controls osmoregulation, motility, and virulence [49–58].,RisA is phosphorylated to signal internally and increase expression of cell-surface antigen genes vraA and vraB, which are still not fully characterized [40,41,59], as well as *vrgs* and genes related to chemotaxis, flagella, iron-regulated genes, and other unknown genes [38,41]. While *risA* is undoubtedly involved in infection [60], the role of *risS* as a histidine kinase appears to be unclear as it appears truncated in some *Bordetella* spp. [41]. And the fact that RisS is not functional in *B. pertussis* has led some authors to consider RisA as an orphan response regulator in *B. pertussis.* However, in 2016, Coutte *et al*. identified RisK and proposed a model in which Bvg and Ris cooperate under the command of cyclic guanosine monophosphate (GMP) or

cyclic-di-GMP [38]. Later, it was discovered that RisK is required for activation of Bvg^--regulated genes by RisA [19].

RisAS appears to play a significant role during *Bordetella* spp. infections as it is required for key biological functions such as respiratory stress and other metabolic processes, suggesting that this TCS might contribute to persistence and chronic infection [48]. RisA is required for the expression of Bvg^- repressed genes *vraA* and *vraB* [41]. RisAS regulates the expression of virulence factors, including *vrg6, vrg18, vrg24, vrg73*, and *VraA/B* [21]. Despite knowing that regulation of *vrgs* expression requires BvgR and RisA [19,38], the mechanism is not fully understood, but there is speculation that it might be regulated by c-di-GMP levels, but exactly how this might work remains undiscovered. Recently a role for RisA in *Bordetella* spp. transmission has also been suggested [38]. There is still more evidence that needs to be gathered to unravel the specific role of this TCS in transmission; however, Bvg^- genes are important for transmission (for example tEPS), and Ris might be jointly regulating the expression of these genes that are Bvg^- and are necessary for what is probably the extra-host phase.

Recently the Ris system has gained more attention from researchers in the *Bordetella* spp. field. The reason why the histidine kinase is different between *B. pertussis* and the other two classical *Bordetella* spp. is unknown, but interestingly *B. pertussis* infects humans exclusively, while the other two species can also infect animals. The Ris system needs to be further explored to find other genes that are regulated, why there are two alternative kinases in two species, or what other regulators can be regulated by this; all its complexity makes it even a more fascinating challenge to uncover.

PlrRS TCS

A third TCS recently discovered in *Bordetella* is *PlrRS* (persistence in the lower respiratory tract, sensor kinase), which is essential for bacterial establishment in the lower respiratory tract [20,37]. The genes involved in this system are a *ntrY*-like sensor kinase, *plrS*, and *plrR*. *plrS* mutants are not able to colonize the lower respiratory tract but can be rescued by wild-type cells. The infection is also cleared more quickly when mice are infected with the deletion mutant of *plrS* [37]. This indicates the ability conferred by *plrS* to combat the host's inflammatory immune response. Again, FHA plays a role in establishing colonization in the lower respiratory tract, highlighting the protein's role as an essential component of *Bordetella* virulence and infection. The PlrRS system responds to CO_2 levels in the environment by differentially regulating gene expression; higher CO_2 levels induce increased virulence [20]. PlrS is an upstream regulator of Bvg and, as such, it also regulates other virulence factors. More studies are necessary to unravel the genes that are under the command of this regulatory mechanism and to discover how the network between PlrRS and BvgAS works.

These three TCSs are still being studied by several groups. The collective knowledge to date is showing an intriguingly complicated and highly sophisticated molecular network that controls gene expression after sensing environmental cues. But, could some of those environmental cues be immune components that are able to be detected by bacteria and, accordingly, affect bacterial gene expression? Since the host immune system presents the greatest threat to bacterial colonization, survival, and persistence, we suggest that these systems contribute to immunomodulation.

Sigma Factors

Bacterial sigma factors can control gene expression by directly binding to the promoter regions of genes. Their activity responds to environmental or developmental signals, changing patterns

of gene expression. There are also anti-sigma factors that control the activity of sigma factors by specifically binding to them and preventing their interaction with the RNA polymerase [61].

σE (RpoE) is a subfamily of sigma factors that are present in many bacteria. RpoE is involved in stress responses to various environmental stimuli, such as disturbances in the outer membrane [26,62–64]. In *E. coli*, the mechanism by which RpoE is activated is mediated by RseA, which is an anti-sigma factor located in the cytoplasmic membrane [65]. The σE in *Bordetella* spp., SigE, was studied first in *B. bronchiseptica*, where the results showed that SigE is involved in cell-envelope stress caused by heat shock, exposure to ethanol and detergent, and specific stresses caused by several β-lactam antibiotics. Importantly, *sigE* was not required for colonization and persistence in the murine model, indicating that it did not have a phenotype that changes its interactions with the host [66].

The anti-sigma factor, RseA, which works in conjunction with RpoE, was first studied using *B. pertussis* as a model. When comparing this mutant strain to the wild-type strain of *B. pertussis*, an *rseA*-deficient mutant presents higher growth at 25 °C and produces more outer membrane vesicles, indicating that it plays a role in membrane maintenance as previously described for other species. Interestingly, the mutant strain of *B. pertussis* lacking *rseA* presented higher amounts of ACT while expressing less pertussis toxin and fewer other virulence factors, indicating its role in controlling virulence factors independently of the Bvg TCS. This was an interesting finding as ACT was the only virulence factor that appeared to increase. When studying its role in host interactions, a deficiency in persistence, in both *in vitro* (J774A.1 macrophages and neutrophils) and *in vivo* experiments (CD1 mice), was reported [67], suggesting that this gene might be involved in the first steps of colonization and adaptation to the host. These results suggest that sigma/anti-sigma factors might be playing a key role in adaptation to host environment by regulating expression of specific virulence factors.

Another sigma/anti-sigma factor in *Bordetella* spp. is BtrAS, which is itself Bvg$^-$regulated and is involved in the regulation of the T3SS. BtrA (anti-sigma factor) specifically binds BtrS (sigma factor). BtrA positively regulates several genes, including those that encode adhesin and toxins (ACT, FHA, fimbria, PRN, and SphB1), negatively regulates *flaA* expression, and represses a subset of genes of the T3SS. The deletion of *btrA* from *B. bronchiseptica* background has no effect on the colonization of the mouse respiratory tract. In *B. pertussis*, BtrA controls T3SS-mediated cytotoxicity [68]. Remarkably, the deletion of BtrA activates expression of homologs of the *B. pertussis* loci that encode pertussis toxin and the type 4 secretion system that exports it [18,68,69]. Similarly, BtrW, BtrV, and BtrU also regulate T3SS, indicating that there is more than one mechanism to regulate the expression of key genes [70].

While we know that sigma factors play an important role in the regulation of the expression of genes related to virulence and host interaction, the intricacies of how they interact with the other bacterial regulatory mechanisms, such as TCS, chaperones, or sRNAs, remain unclear.

Other Regulatory Mechanisms in *Bordetella* spp.

In addition to the TCS and sigma factor systems, other regulatory mechanisms exist in *Bordetella* spp. Hfq, a chaperone, is involved in virulence, interacting with regulatory sRNAs and facilitating their antisense interaction with targets. As such, Hfq plays an important role in many cellular processes in bacteria, responses to stress, and regulation of virulence factors

[71]. BtcA and Btc22 (BB1618) have been shown to be involved in the regulation of the T3SS [72,73]. BtcA interacts with BteA, which is responsible for the cytotoxicity of *Bordetella* spp. [74,75]. Btc22 is involved in secretion and stability of Bsp22 [73]. The membrane-associated chaperones related to protein secretion include DegP and Par27. DegP (also known as HtrA), which degrades misfolded proteins [76], rescues unfolded proteins, assembles the β-barrel outer membrane proteins [77], and extends FHA polypeptide in the periplasm [78]. DegP was also shown to facilitate the periplasmic transit of the FHA precursor [79]. Similarly, Par27 functions as a periplasmic chaperone and presents high affinity for proteins rich in amphipathic β structure, including FHA [80]. FimB is also a chaperone required for the biogenesis of FHA and the fimbriae [81]. In addition, sRNAs have been detected in *B. pertussis*; however, neither the chaperones nor the sRNAs have been explored in detail as research is still in the early stages.

Currently, there are no described complex regulatory networks explaining the interactions between systems, such as those in *P. aeruginosa* [82], where a well-tailored regulatory network dictates the most suitable phenotype to adapt to specific environments. One example of an opportunity to broaden our understanding of *Bordetella* spp. is in the interaction of the BvgAS and the PlrRS TCS, or RisAS and BvgAS TCS [41] where the subtle mechanisms of this regulation remain unclear. This indicates that there is still more research to be done to understand how *Bordetella* spp. sense environmental cues and to uncover multiple cascades of reactions that could differentially control gene expression to enable bacteria to succeed in and out of the host. What specific TCSs and other regulatory mechanisms are involved in sensing and responding to host immune surveillance? What specific host molecules trigger a response? What molecules are recognized by specific systems? Are different molecules causing different responses utilizing the same pathway?

All of this reveals that there are key pieces of information that need to be put together to understand the molecular bases of the pathways involved in regulating bacterial behavior and adaptation to host immune signals. This will assist us to better design more targeted therapies and vaccines that disrupt the signals that bacteria might utilize to manipulate or evade immunity.

Bordetella spp. Can Manipulate Host Immunity

One of the most important evolutionary pressures for bacterial pathogens is the host immune response. The ability to detect cues of host surveillance and respond to them provides a great fitness advantage. *Bordetella* spp. have developed a set of tools that enable them to successfully manipulate or evade host immunity, guaranteeing longer persistence. This provides a great model to understand persistence and chronic diseases and to study bacterial ability to disable the immune system by altering immune signaling pathways in a natural host–pathogen setting.

Several pathogenic species, including *Bordetella* spp., have been models in studies on how pathogens can escape [83,84] and/or manipulate host immunity (Figure 2; Figure 3, Key Figure). *P. aeruginosa* [85–87], *Neisseria gonorrhoeae* [9,88], and many others [89] have also been extensively studied in regard to their ability to manipulate the host response as well as the molecular pathways that enable them to coordinate their behavior. However, the animal models for some of those species are not in the context of the natural host–pathogen interaction. The unnatural models are restricted and they do not allow an in-depth understanding of the cross-signals between both. Mice and rabbits are natural hosts of *B. bronchiseptica*, allowing for the study of host–pathogen interactions in a natural infection model, but mice have the distinct

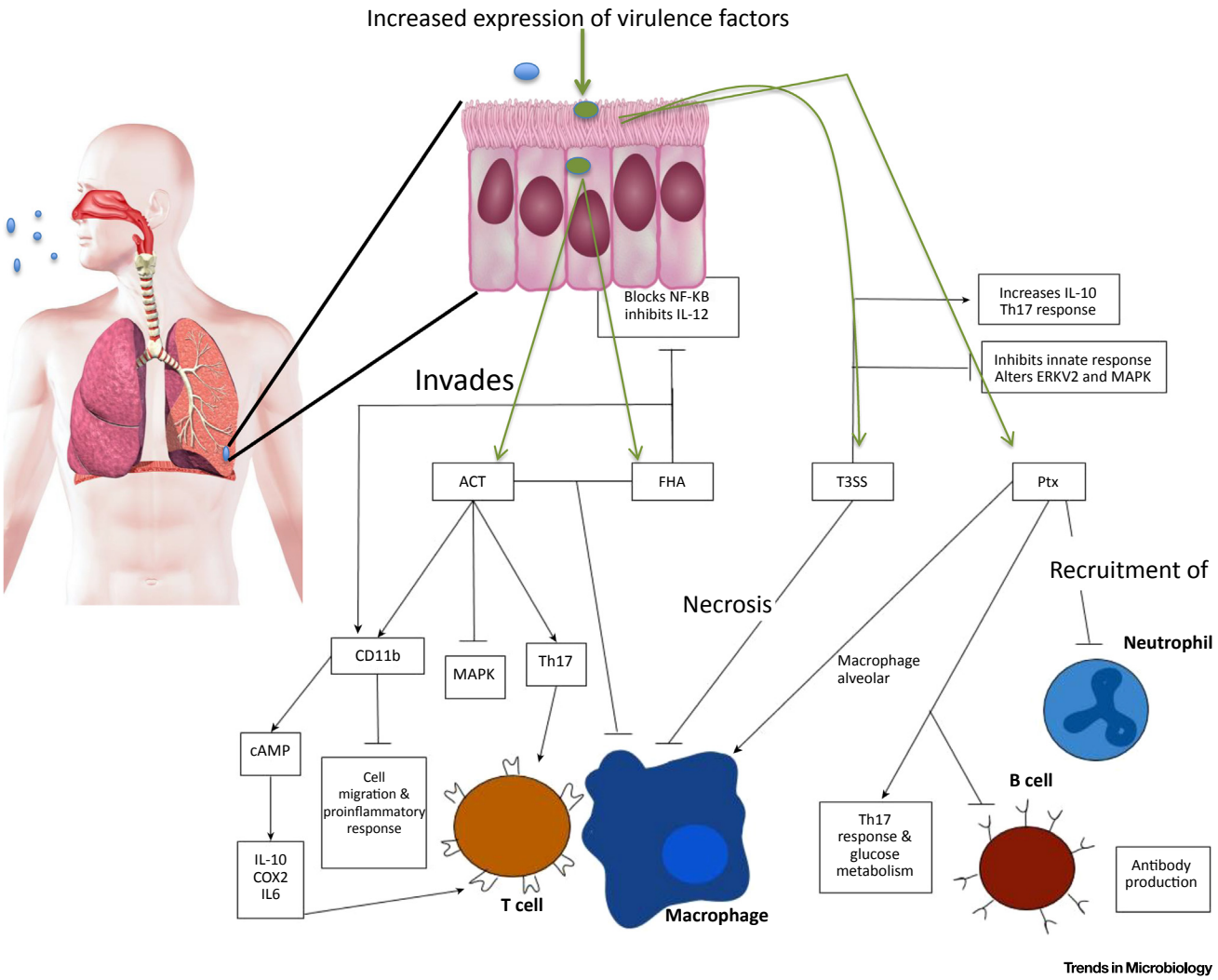


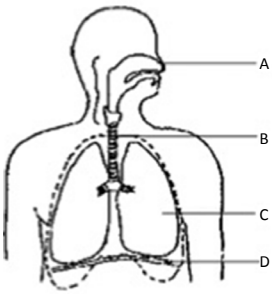
Figure 2. Schematic of the Known *Bordetella* spp. Virulence Factors, and How These Manipulate Host Immune Response. It shows how *Bordetella* changes gene expression (from blue to green), increasing expression of molecules (in black boxes) that downregulate immune response.

advantage of the breadth and depth of immunological tools to study the host side of the interaction.

Amongst the several virulence factors that *Bordetella* spp. utilizes are ACT, PTX, T3SS, and FHA. ACT binds to CD11b/CD18, Mac-1 present in macrophages, dendritic cells, and neutrophils [90,91], forming pores that alter the intracellular calcium levels and subsequently cellular functions [91–96], and also suppressing T cells [97]. PTX induces lymphocytosis [98], inhibits neutrophil recruitment [99–101], and decreases cytokine chemokine production [100,101]. The T3SS modulates cell migration as well as IL-10 production [102]. FHA also alters IL-10 production [103] and suppresses IL-17 [104] and, by altering inflammation, FHA facilitates colonization of the respiratory tract [17]. All of these virulence factors are upregulated in the presence of host cues, such as CO_2 levels, catecholamines, iron starvation, blood, or serum – indicating that the levels of expression increase in response to the host cues in order to

Key Figure

Different Host Microenvironments of *Bordetella* spp.



	Gases	T°(°C)	Cellular components	Microbiota	Immune response	Refs
(A) Nasal cavity	78.09% N_2 20.95% O_2 0.04% CO_2	30–35 (nose)	Olfactory epithelium (olfactory sensory neurons, glial cells, sustentacular cells, microvillar cells, basal cells, brush cells, Bowman's glands)	Many bacterial species	Variable inflammation, mucosal immunity	[31,53,56, 68,88,93]
(B) Trachea	Decreased O_2 Increased CO_2 Decreased H_2O	37	Connective tissues and muscles Epithelium composed of goblet cells and ciliated cells Variable blood supply Hyaline cartilage	Lower diversity	High level of mucus (inorganic salt, antiseptic enzymes, immunoglobulins, glycoproteins, mucins)	[22,25, 31,53,56, 68,88,93]
(C) Lung	5% CO_2 High reactive oxygen species Gas exchange	37	High levels of serum Lung epithelium (goblet cells, basal cells, pseudostratified columnar cells, cuboidal cells, squamous cells) High levels of blood supply and high content of iron-containing red blood cells	Lower diversity	High levels of inflammation Innate and adaptive responses Alveolar macrophages Low levels of mucus	[22,25, 31,53,56, 68,88,93]
(D) Blood (deeper tissues)		37	Blood and serum (into deeper tissues) Blood (45% erythrocytes, 54.3% plasma [including thrombocytes], 0.7% leukocytes) Albumin, globulin, fibrinogen, neutrophils, monocytes, lymphocytes, basophils, eosinophils, salts, thrombocytes, water, O_2, CO_2, N_2, hormones, enzymes	Lower diversity	High levels of inflammation. Innate and adaptive responses. Antibacterial components, complement, host molecules, cytokines	[68,128]

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Figure 3. For a Figure360 author presentation of Figure 3, see the figure legend at https://doi.org/10.1016/j.tim.2018.09.010.
This figure indicates the different microenvironments within the host respiratory tract that *Bordetella* encounters and adapts to. Highlights of the different conditions are shown. See also [22,25,31,53,56,68,88,93,128].

enable bacteria to manipulate host immunity and allow for colonization, persistence, reinfection, and transmission.

Altogether, this indicates that *Bordetella* spp. virulence factors interfere with host immune signals, causing the host to induce an anti-inflammatory response that ultimately leads to evasion of the host immune response resulting in the establishment of infection and an increase in bacterial persistence. *Bordetella* spp. offer a great opportunity to understand in detail how these virulence factors are regulated and information is incorporated about the manipulation of the host response. Understanding bacterial immunomodulatory pathways offers the opportunity to develop new strategies for vaccine and therapeutic development.

Concluding Remarks

Bordetella spp. offer a great opportunity to understand in detail how these virulence factors are regulated, and how they manipulate host response. Some mechanisms that *Bordetella* spp. utilize to manipulate host immunity are well known, but how *Bordetella* spp. sense environmental cues, or how they adapts to them, is not fully understood (see Outstanding Questions). We do not fully comprehend how *Bordetella* spp. regulate gene expression or how connections between TCSs, sigma factors, sRNAs, or chaperones modulate gene expression that allows the bacteria to ultimately hijack the host immune response. Using *Bordetella* spp. to understand how bacteria sense immune surveillance, and how they activate the expression of genes that counteract and manipulate immunity, will allow for the design of better vaccines and/or therapies that restrain the ability of microorganisms to manipulate the host.

Outstanding Questions

What specific host signals can *Bordetella* spp. sense?

How do *Bordetella* spp. regulate/activate the expression of sigma factors, sRNAs, or chaperones, and what genes do they control?

How are all the regulatory mechanisms (TCSs, sigma factors, chaperones, and sRNAs) interconnected in *Bordetella* spp.?

Do different host signals induce different bacterial responses?

Do other bacteria respond to analogous cues in their microenvironment?

Can inhibitors that impede sensing be effective therapeutics?

Can we interrupt these pathways to attenuate pathogens, creating safe live vaccines and/or probiotic benign competitors?

Acknowledgments

The authors would like to thank first of all Dr Amy Medlock for all the great discussions and suggestions that enriched this manuscript. Thanks to Jonathan Gurr for the edits that have improved this manuscript. Also, thanks to all members of the Harvill laboratory for their comments and discussions. This work was supported by National Institutes of Health (NIH) (grant numbers GM083113, AI107016, AI116186, GM113681 to ETH), and Georgia Clinical and Translational Science Alliance (grant number 1UL1TR002378-01). The funders had no role in study design, data collection, and interpretation, or the decision to submit the work for publication.

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