

***Bordetella* evolution: lipid A and Toll-like receptor 4**

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The evolution of *Bordetella pertussis* and *Bordetella parapertussis* from *Bordetella bronchiseptica* involved changes in host range and pathogenicity. Recent data suggest that the human-adapted *Bordetella* modified their interaction with host immune systems to effect these changes and that decreased stimulation of Toll-like receptor 4 (TLR4) by lipid A is central to this. We discuss *Bordetella* lipid A structure and genetics within the context of evolution and host immunity.

Keywords: *Bordetella*, evolution, lipid A modification, Toll-like receptor 4

The bordetellae

The genus *Bordetella* contains nine species, three of which have been studied in detail: *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*. These three species have different host ranges and cause different diseases in their hosts. *B. pertussis* causes whooping cough predominantly in children aged 1–10 years and is recognised as a cause of persistent cough in adults.^{1–5} *B. parapertussis* also causes whooping cough in children, but disease due to *B. parapertussis* has often been regarded as milder than that caused by *B. pertussis*.⁶ *B. parapertussis* has evolved as two distinct lineages, one adapted to the human host and the other adapted to ovine hosts.^{7–10} In contrast to the severe restriction in host range exhibited by *B. pertussis* and *B. parapertussis*, *B. bronchiseptica* infects a wide variety of mammals, although infections in humans are rare. Although described as a pathogen, *B. bronchiseptica* has only been reported to cause disease in a small number of hosts. This includes infectious tracheobronchitis in dogs (kennel cough) and cats, atrophic rhinitis in swine and bronchopneumonia in rabbits and

other laboratory animals.^{11–14} It is likely that, in most hosts, *B. bronchiseptica* infection is asymptomatic although it is likely that this can be altered by factors including stress and secondary infections.

The differences between the host ranges and diseases of the Bordetellae are intriguing given that the principles underlying the pathogenesis of these bacteria are very similar, as reviewed by Cotter and Miller.¹⁵ *Bordetella* are acquired through infected droplets from other hosts. They display a strong tropism for the cilia of the respiratory mucosa and this represents the major, if not the only, site of infection for these bacteria.^{16,17} Colonisation is followed by proliferation on the ciliated mucosal surface resulting in ciliostasis, damage to the respiratory epithelium, induction of mucus release and an inflammatory influx into the lumen of the respiratory tract.^{16,17} Abrogation of normal ciliated mucosal function and damage to the respiratory epithelium is the primary pathology associated with many *Bordetella* infections. Although *B. pertussis* and *B. parapertussis* are not natural pathogens of mice, they colonise the mouse respiratory tract, proliferate in this niche and induce a host immune response when administered experimentally; thus, the mouse model provides a tool to probe the infection biology of the Bordetellae, as discussed by Mattoo and Cherry.¹⁸

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Evolution of B. pertussis and B. parapertussis

B. pertussis and *B. parapertussis* evolved from *B. bronchiseptica* (or a *B. bronchiseptica*-like organism) through

genome reduction and re-arrangement.^{19,20} There are no *B. pertussis* or *B. parapertussis*-specific genes identified to date suggesting that loss of genes and changes in expression patterns of common genes has driven the changes in their host range and pathogenesis.^{21,22}

The evolution of any bacterium is shaped by the selection pressures faced by the organism. A recent article argues that *B. pertussis* and *B. parapertussis* faced selection pressures for a change from chronic to acute infections and for avoidance of existing host immunity during their evolution.²³ The evolution of species towards causing highly contagious infections, but with a low infectivity period, from a progenitor that is much less contagious but with a very prolonged infectious period, is a recurrent theme in the evolution of human pathogens.²⁴ Pathogens of hosts that are sparsely populated tend towards chronic infections that permit colonization of the host for the long periods between encounters with other susceptible hosts. Many of these pathogens also survive in the environment, facilitating acquisition by other susceptible, mobile hosts.

The presence of hosts that live in dense populations means that encounters between such hosts are frequent. This favours acute infections producing symptoms (such as coughing and sneezing) that facilitate the spread of bacteria directly between hosts and eliminates the need for prolonged survival in the environment. Urbanization of human populations took hold during the Middle Ages and coincides with the likely emergence of whooping cough as it was first clearly reported in the 17th century.²⁵ The loss of an environmental phase from the life cycles of *B. pertussis* and *B. parapertussis* probably explains much of the genome reduction that has occurred. Many of the genes lost from these two species are predicted to encode surface components in *B. bronchiseptica*.¹⁹ It is likely that these are involved in interactions between *B. bronchiseptica* and its environments, both inside and outside of its different hosts. Restriction of *B. pertussis* and *B. parapertussis* to the human host is, therefore, likely to have rendered many of these genes obsolete and allowed for their deletion, or even possibly favoured their deletion due to 'streamlining' of their genomes.²⁰

This genetic reduction does not explain their evolution towards causing acute infections and the genetic basis for this is less clearly defined. However, recent studies have indicated that the *Bordetella* interact differently with their hosts and that this may contribute to the different nature of their infections. For example, pertussis toxin (PT) expression has been detected only in *B. pertussis*.²⁶ It has been shown recently to inhibit neutrophil recruitment into the respiratory tract to delay antibody-mediated clearance of *B. pertussis* in a mouse model of infection and thus perhaps contribute to the acute nature of *B. pertussis* infections.^{27,28} Other species-specific gene

expression patterns have been identified and are likely to be important contributors to the different host ranges and diseases observed with these bacteria.

Toll-like receptor 4 (TLR4) in Bordetella immunity

Another interesting difference is the role for the host molecule, TLR4, in protecting the host from infection by different *Bordetellae*. TLR4-deficient mice rapidly die following inoculation with as few as 1000 *B. bronchiseptica* whereas the congenic wild-type mice withstand inoculation with more than 10⁶ bacteria with no observable signs of infection.²⁹ A TLR4-dependent, transient expression of tumor necrosis factor (TNF)- α is required for protection.³⁰ Interestingly, although TLR4 is required for mice to control *B. pertussis* infection, this bacterium does not rapidly kill TLR4-deficient mice.^{31,32} They display a limited defect that is only evident after the first week of infection with *B. pertussis*.^{31,32} TLR4-deficient mice show no apparent differences from wild-type mice in response to infection with *B. parapertussis*.³² This correlates with *B. bronchiseptica* lipopolysaccharide (LPS) displaying 10- and 100-fold greater stimulation of TLR4 *in vitro* than *B. pertussis* or *B. parapertussis* LPS, respectively.^{29,32} These studies argue that, in adapting to infect humans, *B. pertussis* and *B. parapertussis* independently modified their LPS to reduce TLR4-mediated responses, which is likely to promote acute, rather than chronic, infections. Our laboratory is investigating the genetic basis for this differential activation of TLR4 by the different *Bordetellae* and its role in infection and immunity.

Bordetella lipid A

The different TLR4 activating properties of the *Bordetella* LPSs correlate with species-specific lipid A structures in these bacteria.³³ *B. bronchiseptica* is the only one to express a hexa-acylated lipid A as a major species, some of which have the '4+2' arrangement of acyl chains that is characteristic of high potency lipid A species (Fig. 1), although tetra- and penta-acylated species are also present.^{33,34} Heterogeneity among *B. bronchiseptica* strains was observed due to both the variable presence of acylation at certain positions and in one strain acylation with either C12 or 3-OH C12 at the 3 position, suggesting that *B. bronchiseptica* lipid A is variable within and between strains.³⁴ In contrast, *B. pertussis* and *B. parapertussis*, whose LPSs are of low potencies, are reported to express mainly hypo-acylated lipid A species (Fig. 1), and this too is in agreement with the general model for structure/function relationships of lipid A/TLR4 stimulation.^{33,35} This difference in acylation might

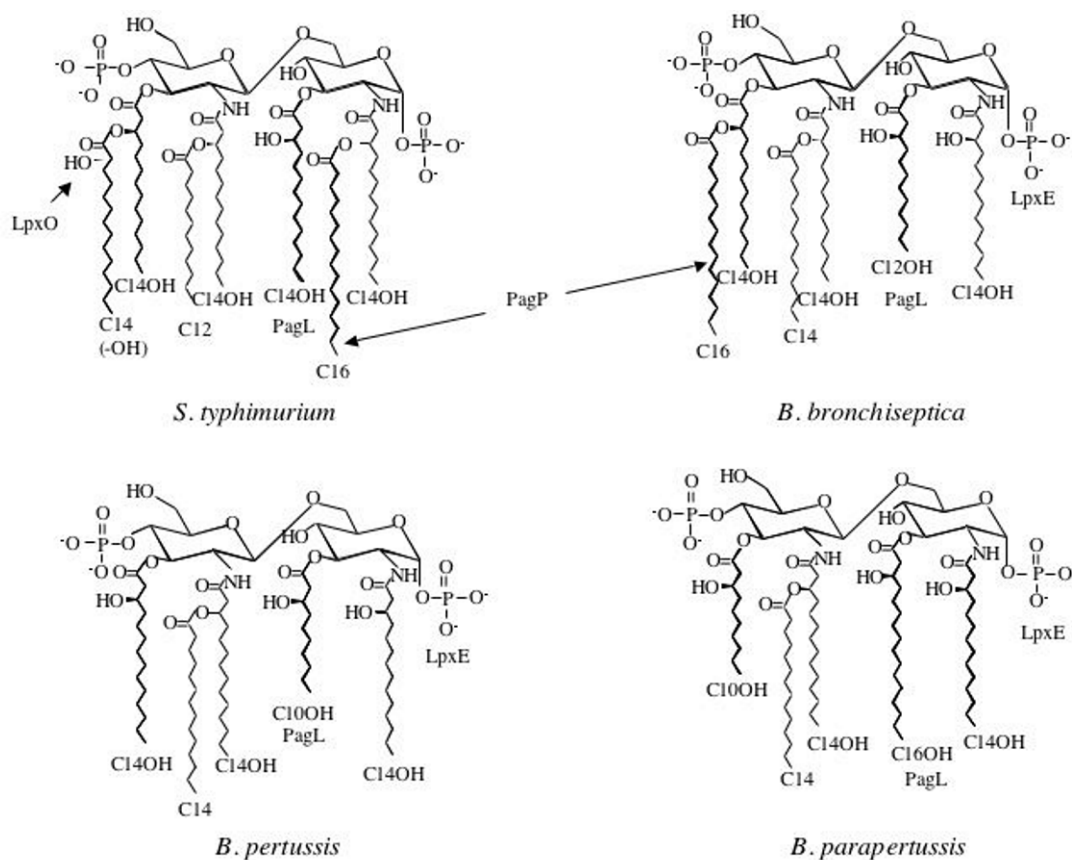


Fig. 1. *Bordetella* synthesise species-specific lipid A structures. Schematic depicting major structures of *Salmonella enterica* sv. Typhimurium and *Bordetella* lipid A species, with the modifications discussed in the text highlighted (not all *Salmonella* lipid A modifications are shown here). PagP: mediates palmitoylation at the 2 position in *Salmonella enterica* sv. Typhimurium and the 3' position in *B. bronchiseptica* (the position of PagP mediated palmitoylation in *B. parapertussis* is unknown); PagL: de-acylates at the 3 position; LpxO: produces 2-hydroxymyristate in *Salmonella enterica* sv. Typhimurium; LpxE: characterised LpxE enzymes dephosphorylate at the 1 position. *Bordetella* contain *lpxO*, *pagL* and *lpxE* homologues but the modifications expected for the function of these genes have not been reported for these bacteria and thus the position of these modifications in *Bordetella* lipid A is based on the characterised activity of the enzymes in other bacteria.

appear to explain the differences in potency of these lipid A species. However, the genetics of *Bordetella* lipid A biosynthesis suggest a complicated scenario.

An unusual feature of *Bordetella* lipid A is that the primary acylations at the 3 and 3' position are different and differ between species.^{34,35} This is, in part, explained by the relaxed substrate specificities of the *Bordetella* LpxA enzymes and suggests that differences in the activities of orthologues between species contribute to their different structures, but the role of this asymmetry in LPS function is unknown.³⁶

Modification of *Bordetella* lipid A

The *Bordetella* genomes contain a number of homologues of lipid A modification genes (Table 1; A. Preston, unpublished observations). Regulated covalent modifications of lipid A were originally characterised in the *Enterobacteriaceae* but have now been identified in

a variety of bacteria.³⁷ We have characterised *B. bronchiseptica* *pagP*, demonstrating that *B. bronchiseptica* lipid A is palmitoylated, that this modification is *pagP*-dependent and that expression of this gene is regulated in response to environmental stimuli.³⁸ Furthermore, we showed that *pagP* is required for persistence of *B. bronchiseptica* within the mouse respiratory tract, through resisting antibody-dependent complement-mediated killing, the first description of a direct role for a *pagP* gene in virulence.^{38,39}

B. parapertussis contains a *pagP* gene identical to that of *B. bronchiseptica* that is required for palmitoylation of its lipid A (R. Ernst, personal communication) and for wild-type levels of endotoxicity (A. Preston, unpublished observations), but its role in *B. parapertussis* virulence has not been tested. Although *B. pertussis* also contains a *pagP* coding sequence identical to that of the other two, a mutation event has deleted the *pagP* promoter and no activity can be detected from *B. pertussis* in *in vitro* assays of PagP activity (A. El Zoebay, R. Bishop and A. Preston, unpublished observations).

Table 1. Putative *Bordetella* lipid A modification genes

	PagP	PagL	LpxO	LpxE
<i>B. pertussis</i>	– (promoter deleted) (BP3006)	– (frame shift mutation) (BP3592)	+	+
			(BP2333)	(BP0835)
<i>B. bronchiseptica</i>	+	+	+	+
	(BB4181)	(BB3771)	(BB3402)	(BB3846)
<i>B. parapertussis</i>	+	+	+	+
	(BPP3735)	(BPP3320)	(BPP1706)	(BPP3396)
Conservation of coding sequences	BB/BPP identical	BB/BPP identical	All 3 identical	BPP:BB/BP I256V substitution

–, gene is predicted to be inactive; +, gene is predicted to be expressed.

Gene number as given in the *Bordetella* genome sequence annotation is shown in parentheses.

The bordetellae also contain a *pagL* homologue. *B. bronchiseptica* PagL has de-acylase activity when expressed in *Escherichia coli* but *Bordetella* LPS de-acylated at the 3-position has not been reported.⁴⁰ Interestingly, *B. pertussis pagL* is disrupted by a frameshift mutation and is thus predicted to be inactive.⁴⁰ The role of *pagL* in *Bordetella* LPS structure or pathogenesis is unknown.

All three *Bordetella* genomes contain as yet uncharacterized *lpxO* and *lpxE* genes.^{41,42} Each appears to be intact. The three *lpxO* genes are identical and among the *lpxE* genes the only difference is a conservative isoleucine for valine substitution in the *B. parapertussis* predicted protein sequence. Interestingly, none of the reported lipid A structures of these bacteria have contained 2-hydroxy fatty acids, whereas these were identified in the lipid A species of the related bacteria *B. trematum* and *B. hinzii*.³³ Thus the role of *lpxO* in *Bordetella* LPS structure and pathogenesis is unknown. Variably phosphorylated lipid A has been reported for *B. pertussis*, although the lipid A analysed in this report had been prepared under dephosphorylating conditions, but the role of *lpxE* in this is unknown.⁴²

The presence of these putative lipid A modification genes in the Bordetellae suggests that the few reported *Bordetella* lipid A structures may not describe the true complexity of these molecules in these bacteria.

However, the differential activation of TLR4 signalling by different *Bordetella* suggests that *B. pertussis* and *B. parapertussis* have altered their lipid A to alter their interaction with this facet of host immunity during their evolution from *B. bronchiseptica*. A recent paper supports this hypothesis.⁴² *B. bronchiseptica pagP* and *pagL* were expressed in *B. pertussis* resulting in palmitoylated and de-acylated lipid A, respectively. The palmitoylated lipid A had increased TLR4 stimulating activity whereas the de-acylated lipid A was decreased

for this activity. Interestingly, both of the modified strains had greater TLR4 stimulating activity than the wild-type strain, which was attributed to PagL-induced release of LPS making it more available to TLR4 than cell-associated LPS. While inactivation of lipid A modification genes might appear to explain the difference between the potencies of *B. bronchiseptica* and *B. pertussis* lipid A species, *B. parapertussis* lipid A is of very low potency but appears to contain the lipid A modification genes intact. Thus, while there are clearly differences between the Bordetellae in their interaction with the TLR4 signalling complex, the precise molecular basis for these differences cannot be defined until the full repertoire of *Bordetella* lipid A heterogeneity has been described.

CONCLUSIONS

The evolution of *B. pertussis* and *B. parapertussis* lipid A offers fascinating insights into mechanisms by which pathogens adapt to new host ranges and to changes in host dynamics by manipulation of the host immunity-pathogen interactions.

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