

# Highlights of the 12th International *Bordetella* Symposium

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To commemorate the 100th anniversary of the Nobel prize being awarded to Jules Bordet, the discoverer of *Bordetella pertussis*, the 12th International *Bordetella* Symposium was held from 9 to 12 April 2019 at the Université Libre de Bruxelles, where Jules Bordet studied and was Professor of Microbiology. The symposium attracted more than 300 *Bordetella* experts from 34 countries. They discussed the latest epidemiologic data and clinical aspects of pertussis, *Bordetella* biology and pathogenesis, immunology and vaccine development, and genomics and evolution. Advanced technological and methodological tools provided novel insights into the genomic diversity of *Bordetella* and a better understanding of pertussis disease and vaccine performance. New molecular approaches revealed previously unrecognized complexity of virulence gene regulation. Innovative insights into the immune responses to infection by *Bordetella* resulted in the development of new vaccine candidates. Such discoveries will aid in the design of more effective approaches to control pertussis and other *Bordetella*-related diseases.

**Keywords.** *Bordetella*; whooping cough; pertussis epidemiology; pertussis vaccines; *Bordetella* genomics.

The 1919 Nobel prize in Physiology/Medicine was awarded to Jules Bordet for his discovery of alexine, today referred to as complement. However, to the *Bordetella* community he is probably best known for the identification of the whooping cough agent, initially named *Haemophilus pertussis* and now known as *Bordetella pertussis*. In honor of Jules Bordet and to commemorate his Nobel Prize, the 12th International *Bordetella* Symposium was held from 9 to 12 April 2019, at the Université Libre de Bruxelles, where he studied and spent most of his scientific career.

## OPENING SESSION

Several grandchildren and great-grandchildren of Jules Bordet were present during the opening session, and Bordet's great-granddaughter Nathalie Devroey presented a portrait of her great-grandfather with focus on his generous and warm personality. She explained how he introduced his grandchildren to scientific discovery and to

classic literature, and how “he had the most marvelous ability to explain the most complicated things in the most understandable ways.”

James D. Cherry delivered the opening lecture on the 112-year odyssey of pertussis and pertussis vaccines. He highlighted the various misconceptions and their consequences for the current pertussis problem. Although on the rise in several countries, the rate of pertussis today is approximately 20-fold less than in the prevaccine era [1]. Furthermore, vaccine failures result in less severe illness than seen in unvaccinated children. Pertussis occurs in cycles of 2–5 years, which has not significantly changed since the introduction of pertussis vaccines, indicating that vaccination does not impede *B. pertussis* circulation. Asymptomatic infections, a major finding in children, are 4–22 times more common than symptomatic infections. Pertussis toxin (PT) is a major virulence factor in clinical disease [2], and an as yet unidentified “cough toxin” may participate in the clinical picture of pertussis. Finally, he discussed reasons why acellular pertussis (aP) vaccines are inferior to whole-cell (wP) vaccines, including linked-epitope suppression, genetic changes induced by aP vaccines, a suboptimal balance of antigens, and failure to induce the appropriate T-cell subtypes. Thus, new pertussis vaccines are needed, either multicomponent aP vaccines with appropriate adjuvants, less reactogenic wP vaccines, or live attenuated vaccines.

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## SPECIAL SESSION ON ADENYLATE CYCLASE TOXIN

A special session was organized as a tribute to the lifetime achievements of Erik Hewlett, who made seminal contributions to understanding the role of adenylate cyclase toxin (ACT) and played a key role in the initiation and organization of many of the previous International *Bordetella* Symposia and in fostering the constitution of the International *Bordetella* Society.

F. Heath Damron summarized Hewlett's career, from his college training in Fulton, Missouri in 1968, to his full professorship at the University of Virginia. He also highlighted Hewlett's scientific contributions to the understanding of the ACT biology and the enhanced protective effect of adding ACT to aP vaccines [3].

Erik Hewlett followed by presenting the history of ACT. A collaboration between Hewlett, Jan Wolff, and Charles Manclark led to the discovery of a 70-kDa extracytoplasmic adenylate cyclase lacking toxin activity [4], which was subsequently shown to be a fragment of the much larger ACT secreted by *B. pertussis*. His team demonstrated that ACT secretion and folding requires calcium, that the expression of its enzymatic activity requires calmodulin, and that ACT interacts with filamentous hemagglutinin and inhibits biofilm formation [5].

Daniel Ladant delivered a lecture in honor of Agnès Ullmann, who had passed away a few months earlier, at the age of 92. He summarized her major contributions on ACT, including the purification of full-length ACT, the cloning and sequencing of its structural gene *cyaA*, and the deciphering of the *cya* locus genetic organization and transcriptional regulation [6]. He then presented the use of ACT as antigen-delivery vehicle and provided examples of ACT-grafted antigens delivered into the cytosol of antigen-presenting cells [7]. He also illustrated the use of ACT as a tool for the study of protein-protein interactions [8]. Finally, he presented a model for ACT translocation into target cells and activation of the enzyme by calmodulin [9].

Peter Sebo illustrated the multiple and sometimes unexpected biological activities of ACT. He showed how calcium drives the formation of an intramolecular Brownian ratchet that directs unfolded ACT through the secretion system, and how ACT interacts with CD11b/CD18 on myeloid phagocytes and is then translocated. ACT blocked phagocytosis and oxidative burst, suppressed Toll-like receptor (TLR) signaling, induced apoptosis, and reprogrammed tolerogenic dendritic cells and macrophages [10, 11].

## CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF PERTUSSIS

Nicola Klein presented data on the epidemiology of pertussis in children and adolescents and noted the rapid loss of effectiveness of the reduced antigen aP vaccine in preadolescents over a 3-year period.

A human challenge model of *B. pertussis* colonization was presented by Robert Read. He described a dose-finding study and found that the dose necessary to achieve 80% colonization was  $10^5$  colony-forming units, and that colonization peaked at day 11 and was associated with serum anti-*B. pertussis* antibody titer rises [12].

Helen Campbell presented an update on the national pertussis immunization program of pregnant women in England and assessed vaccine effectiveness (VE). Vaccine coverage was 72% in 2017–2018, and VE in infants  $\leq 2$  months of age was 90% to 97% against fatal infant pertussis. The VE of 5-component aP vaccine was greater than that of 3-component aP vaccine. Using hospital-based surveillance in Australia, Helen Marshall reported 85% VE of maternal immunization for infants aged  $\leq 2$  months and 70% VE for infants  $\leq 6$  months. Among 183 infants suffering from pertussis, 16% required intensive care, 6% required assisted ventilation, and 1 infant died.

Matt Edmunds presented a retrospective South England secondary boarding school cohort study and showed that 30% had culture-confirmed, probable, or possible pertussis; 12% had asymptomatic infections; and 6% were asymptomatic carriers. The overall attack rate was 48%. However, nonreporting was a problem, and he suggested that aP vaccination should be used in outbreak situations and routine adolescent aP boosters should be considered.

René Raeven compared anti-PT immunoglobulin G (IgG) values and immunoproteomic profiles of *B. pertussis* infection-induced responses between humans with different pertussis immunization backgrounds (aP vs wP). He used multiplex immunoassays, 1- and 2-dimensional immunoblotting, and mass spectrometry, both to the usual *B. pertussis* antigens and to outer membrane vesicles (OMVs), and noted that the antibody patterns were different among the groups receiving different pertussis vaccines.

Norman Fry commented on external quality assessment for the detection of *B. pertussis* by polymerase chain reaction (PCR) in Europe. Investigators from Sweden, Finland, and England evaluated different PCR methods and compared results at 2 different times, from 28 different European laboratories. They demonstrated substantial improvement from the first to the second survey.

## BORDETELLA BIOLOGY AND PATHOGENESIS

New information was provided on gene regulation in *Bordetella*, including new data on regulation by the 2-component systems BvgAS and RisAK [13], and by small noncoding RNA. In the virulent phase, the *B. pertussis* BvgAS system is activated, and expression of virulence-activated genes increases. In contrast, their expression decreases in the avirulent phase when *B. pertussis* expresses virulence-repressed genes (*vrgs*). The expression of these genes depends on the transcription factor RisA,

which is phosphorylated by the histidine kinase RisK. Qing Chen investigated whether RisR, whose gene is co-transcribed with *risK*, may also participate in *vrg* regulation. A *risR* deletion was lethal in the Bvg<sup>-</sup> phase. Under Bvg<sup>-</sup> conditions, the *risR* mutant grew only in the presence of suppressor mutations that resulted in a phase-locked Bvg<sup>+</sup> phenotype or mutations that reduced RisA levels. A *risA/risR* mutant rescued the lethality of the *risR* mutation. Whereas the RisA histidine kinase RisS is inactive in *B. pertussis*, it is intact in *Bordetella bronchiseptica* and functions as a phosphatase. Alterations of threonine 262 converted it to a kinase and induced a mucoid phenotype due to the expression of capsular polysaccharide genes.

Gyles Ifill generated an RNAase III active site mutant and an RNase E C-terminal deletion, and observed that half of the differentially expressed genes in the mutants were also found in the transcriptome of an *hfq* mutant published previously [14]. Regulatory RNAs may modulate virulence, as approximately 50% of the differentially expressed genes in the RNase mutants were BvgAS regulated.

Tod Merkel showed that the baboon *B. pertussis* challenge model recapitulates the characteristic signs of human pertussis, including leukocytosis, paroxysmal coughing, mucus production, and *B. pertussis* transmission [15]. It also showed more severe disease in younger than in older animals. However, unlike in young humans, there was no significant pathology in the trachea of baboons. RisA-deficient *B. pertussis* grew well in baboons but was impaired in transmission, indicating that *vrgs* participated in *B. pertussis* transmission. Thibaut Naninck followed *B. pertussis* colonization of the baboon respiratory tract over time by probe-based confocal laser endomicroscopy using green fluorescent protein-expressing bacteria. He could quantify bacterial colonization and observe interactions of the bacteria with antigen-presenting cells in trachea and bronchi.

Using an infant mouse model, Karen Scanlon elucidated the role of PT in the development of severe pertussis. PT activated the pulmonary angiotensin (AT) system and inhibited the protective AT<sub>2</sub> functions in infant mice, eventually leading to pulmonary hypertension. She also showed that angiotensin-converting enzyme inhibitors might be useful to improve disease outcome. Katharina Ernst showed that host cell cyclophilin isoforms CypA and Cyp40 are required for translocation of the PT S1 subunit into the cytosol, which might make them a target for novel therapeutic strategies against pertussis.

Françoise Jacob-Dubuisson presented studies on *B. pertussis* homeostasis of copper, a metal that is both essential and toxic for living organisms. Unlike for other pathogens, copper export or detoxification systems are absent or not copper-regulated in *B. pertussis*. In excess copper, only a cytoplasmic copper chaperone was induced. Under copper starvation, *B. pertussis* upregulated an operon coding for a putative TonB-dependent transporter and 2 proteins of unknown functions. Thus,

*B. pertussis* copper homeostasis is more focused on harvesting the metal than defending from its toxicity.

Iain MacArthur reported that the *B. pertussis* growth inhibition by hydrophobic molecules [16] is due to alterations of the Acr system, an export system of acriflavine and other small hydrophobic molecules. Mutations in *acr* are conserved in *B. pertussis*, suggesting a selective advantage for these mutations. However, neither *Bordetella parapertussis* nor *Bordetella holmesii*, also human pathogens, have these mutations. The selective advantage in becoming more sensitive to hydrophobic molecules thus remains an open question.

## IMMUNITY AND VACCINATION

Kingston Mills demonstrated that *B. pertussis* infection induces interleukin 17 (IL-17)- and interferon gamma (IFN- $\gamma$ )-secreting tissue-resident memory T cells (T<sub>RM</sub>) in the nose and lungs of mice [17], which proliferate after reinfection, mediating rapid pathogen clearance. wP vaccines also primed T<sub>RM</sub>, whereas aP vaccines did not and failed to protect against nasal colonization. An aP vaccine with a TLR2 agonist and c-di-GMP adjuvant instead of alum induced T<sub>RM</sub> cells and opsonizing antibodies. Using PT-deficient *B. pertussis* strains, Cyrille Mionnet found that PT was required to generate mouse lung CD4<sup>+</sup>, CD8<sup>+</sup>, and  $\gamma\delta$  T<sub>RM</sub> cells. Coadministration of PT with the PT-deficient strain restored the generation of T<sub>RM</sub> cells. Adoptive transfer of T<sub>RM</sub> cells protected mice against *B. pertussis* infection.

Jeremy Ardanuy found that the type I and III interferon (IFN) receptors are important activators of inflammatory responses in *B. pertussis*-infected mice. Type I and III IFNs were produced in the lungs 4 days postinoculation and peaked at day 10. Infected type I IFN receptor knockout mice had lung pathology levels similar to those of wild-type mice because of increased levels of type III IFN.

Monica Cartelle Gestal identified a putative regulator, BtrS [18], implicated in the control of immunomodulation. BtrS was involved in survival within macrophages, inflammasome activation, suppression of immune cell recruitment, and persistence in the respiratory tract. Infection with a *btrS* mutant induced robust immunity against reinfection with all classical *Bordetella*.

Michel Kroes demonstrated that activation of human NK cells by *B. pertussis* is secondary to inflammasome activation and interleukin 18 secretion by macrophages. NK cells then secreted IFN- $\gamma$ , which enhanced antimicrobial activity of macrophages and participated in Th1 skewing.

Ricardo da Silva Antunes compared T-cell responses induced after aP boosting of adults vaccinated as infants with aP vs wP vaccines [19]. T cells remained polarized as a function of their original priming. The capacity of memory CD4<sup>+</sup> T cells to expand after boosting was only observed in wP-primed

individuals. Priming by either vaccine was associated with increased antibody titers and plasmablasts after boosting, suggesting that waning immunity is T-cell specific rather than B-cell specific.

Parul Kapil examined whether aP-primed Th2 responses could be polarized towards Th1 and Th17 responses in baboons after *B. pertussis* infection and found that infection of the aP-primed animals induced Th17 and Th2, but no Th1 responses. Following a second challenge with *B. pertussis*, unvaccinated convalescent animals were protected from colonization, while aP-vaccinated convalescent animals were not.

Bahaa Abu-Raya showed that maternal immunization during pregnancy interfered with the infant's antibody production. This was noted both after primary vaccination and after booster doses, and was also seen for antibody titers against diphtheria toxin and pneumococcal polysaccharides, but not for anti-tetanus toxin responses.

Dylan Boehm compared nasal diphtheria, tetanus, and acellular pertussis (DTaP) and DTaP plus curdlan administration in mice and found that in the absence of curdlan, DTaP accumulated in the lungs, whereas in its presence, DTaP was retained in the nasal cavity. Intranasal immunization with either vaccine decreased the bacterial burden in the lungs and induced systemic anti-PT IgG responses together with inhibition of leukocytosis after *B. pertussis* infection. Immunoglobulin A (IgA) was detected in nasal lavages, especially following immunization with DTaP plus curdlan.

Camille Locht explained the rationale for the live attenuated nasal pertussis vaccine BPZE1 [20]. BPZE1 protected against lung and nasal *B. pertussis* colonization in mice and baboons. Protection depended on secretory IgA and IL-17-secreting T<sub>RM</sub> cells [21]. Clinical trials in humans were performed that demonstrated safety of the vaccine and up to 100% seroconversion.

Ang Lin showed that plasmablasts and Th1-type follicular T lymphocytes appeared in BPZE1-vaccinated volunteers 4–14 days after vaccination, as well as BPZE1-specific Th1-polarized T cells. All BPZE1 recipients developed specific memory B cells and anti-BPZE1 IgG and IgA titers. Antigen specificity of these antibodies was broader than that of aP-vaccinated subjects and was predominantly directed to antigens not present in aP vaccines.

Daniela Hozbor illustrated that *Bordetella* OMVs [22] induced superior immunity in mice than aP vaccination. Maternal immunization did not inhibit OMV-induced immunity. *Bordetella parapertussis* and *B. bronchiseptica* OMVs protected against the respective pathogens. Protection depended on lipopolysaccharide O antigen and was also seen with OMVs from bacteria in the avirulent phase.

Jennifer Maynard demonstrated that humanized anti-PT monoclonal antibody hu1B7 [23] provided protection against pertussis in pertussis-challenged neonatal baboons by inhibiting intracellular PT trafficking. A modified version of hu1B7 with

extended half-life was also protective, and white blood cell levels in infected baboons were inversely correlated with hu1B7 levels, which may help in defining minimum protective titers.

## EVOLUTIONARY AND GENOMIC ASPECTS OF *BORDETELLA*

Michael Weigand presented novel approaches to analyze genome diversity including >3500 coding regions of *B. pertussis*. Whole-genome multiple locus sequence typing could also be directly applied on nasopharyngeal specimens.

Andrew Preston revealed that intragenomic recombination between insertion sequences caused genomic rearrangement and that different genome orders exist among *B. pertussis* isolates [24]. Insertion sequence-mediated recombination can result in duplication, with 12 such duplications described. They can be highly unstable [25, 26]. Among 2430 *B. pertussis* isolates analyzed, 191 contained duplications, 94% of which were in 11 "hotspot" loci.

Pertussis has been treated with macrolides for decades, and Qiushui He explained that macrolide-resistant isolates are now found in China [27], as well as sporadically in Europe [28], the Middle East [29] and the United States [30]. Resistance was generally attributed to a A2047G mutation in the domain V of the 23S ribosomal RNA gene. The frequency of this mutation increased in China and is currently at 60%–92%.

By harnessing whole-population and age-stratified incidence data, genomic, and demographic data and vaccine coverage, Ana Bento and Pejman Rohani formulated evolutionary models of *B. pertussis* history and transmission dynamics. They concluded that there was a positive association between genetic diversity and pertussis disease incidence, suggesting that the resurgence is associated with the expansion of strains that cause more detectable symptoms.

## *BORDETELLA* OTHER THAN *BORDETELLA PERTUSSIS*

Eric Harvill discussed *Bordetella* phylogenetics. He highlighted evidence for an environmental origin of the genus, leading to 2 independent but intersecting transmission cycles for *B. bronchiseptica*, 1 circulating as a respiratory pathogen in mammals and 1 as an association between the bacteria and predatory amoeba. As amoeba can colonize mammalian hosts, he speculated that *B. bronchiseptica* can switch between those 2 life cycles [31]. Interactions with amoeba may also represent the starting point for bacterial adaptation to eukaryotic cells. *B. pertussis* evolution toward a closed human-to-human lifecycle has involved genome reduction and the loss of ability to utilize amoeba as reservoir.

Tracy Nicholson characterized the *B. bronchiseptica* BvgR and RisAS regulons by using various isogenic mutants and measuring transcriptional responses of each mutant under

modulating and nonmodulating growth conditions. This revealed a previously unrecognized complex regulation of virulence pathways in *B. bronchiseptica*.

Kacey Yount demonstrated that *Bordetella* colonization factor A (BcfA) [32], used as an adjuvant, enhanced immune responses to *B. bronchiseptica* and *B. pertussis* antigens. Vaccines formulated with BcfA induced protection against *B. bronchiseptica* in mice. With BcfA-containing formulations, the Th2 responses elicited by *Bordetella* immunogens were attenuated, resulting in Th1/Th17 polarized responses.

## CONCLUSIONS

Since the 11th International *Bordetella* Symposium in 2016 [33], the *Bordetella* research community has grown substantially, and the 12th symposium gathered more delegates than ever before.

New, up-to-date technologies were applied to further our understanding and revise some of the previous assumptions about *Bordetella* biology, genomics, immunology, and vaccine development. The baboon challenge model for pertussis was implemented in several laboratories and helped to refine our knowledge on *B. pertussis* infection and vaccine-induced immunity. Particularly exciting was the establishment of a controlled human challenge model for pertussis, which may be helpful to determine correlates of protection and to test novel pertussis vaccines. These advances provide hope for improved control of *Bordetella* infections. It will be interesting to see how these developments have evolved at the 13th International *Bordetella* symposium, scheduled for 2022 in Vancouver, Canada.

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