The role of *B. pertussis* vaccine antigen gene variants in pertussis resurgence and possible consequences for vaccine development.

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Abstract.

Whooping cough, or pertussis, caused by *Bordetella pertussis* is considered resurgent in a number of countries world-wide, despite continued high level vaccine coverage. Among a number of causes for this that have been proposed, is the emergence of *B. pertussis* strains expressing variants of the antigens contained in acellular pertussis vaccines; i.e. the evolution of *B. pertussis* towards vaccine escape. This commentary highlights the contradictory nature of evidence for this but also discusses the importance of understanding the role of *B. pertussis* adaptation to vaccine-mediated immune selection pressures for vaccine-mediated pertussis control strategies.

Resurgence.

Whooping cough, or pertussis, caused by *Bordetella pertussis* is considered resurgent in a number of countries despite high levels of vaccination of infants in these regions. [1] This has involved an increase in the overall number of

cases diagnosed and also a clear increase in disease incidence in older children and adults suggesting a changing epidemiology of pertussis.

Explanations for this recent rise in disease incidence have been reviewed extensively during the last few years (for example, see ref. [2]) but briefly, a switch from the use of a whole cell pertussis vaccine (WCV) to an acellular one (ACV) appears to be a key factor. In humans, the duration of protective immunity afforded by the ACV appears to be shorter than that arising from use of the WCV. [3-5] It has been long recognised that while WCVs induce a mainly Th1 biased response, the response to ACVs has a distinct Th2 bias. [6] Using an infant baboon model of pertussis, there are clear differences between WCV- and ACV-induced immunity. Both types of vaccines protect the individual against disease but animals vaccinated with ACV were colonised to a higher level and for longer when subsequently challenged with *B. pertussis*, compared to animals vaccinated with WCV. [7] Interestingly, vaccine-induced immunity, either from WCV or ACV, was inferior to immunity arising from natural infection in terms of protecting against colonisation following subsequent challenge. [7] Thus, it is very likely that the switch to the use of an ACV has changed the epidemiology of pertussis, resulting in the changing disease patterns observed.

This has led to debate about the need for novel pertussis vaccines, particularly one that induces sterilising immunity. [8] However, there is no clear pathway to testing or licensing such vaccines. It may be possible to introduce novel vaccines as boosters for adolescents and adults but there are major

obstacles to implementing novel vaccines for infant immunisation while the current ones do provide significant protection to infants against disease.

Evolution towards vaccine escape.

Also implicated in resurgence is the evolution of *B. pertussis* such that current strains are less susceptible to vaccine-induced immunity compared to older strains, i.e. *B. pertussis* is evolving towards vaccine escape. [9, 10] There is a clear rationale for why this might happen. The WCV presents a wide range of antigens to the immune system, compared to the 5-9 *B. pertussis* proteins that comprise ACVs. Thus, ACV-induced immunity is focused on just a few proteins, creating a stronger selection pressure for strains expressing antigenic variants of these proteins. Genomic analyses of *B. pertussis* has revealed that recent evolution of these bacteria has involved selective sweeps in which a novel vaccine antigen allele arises and largely replaces the previous dominant allele in the *B. pertussis* population, suggestive of a fitness advantage conferred by the new allele, for example see refs. [11-13]

In particular, regions experiencing resurgence have reported a dramatic rise in the frequency of strains that are deficient in the expression of Pertactin (Prn), one of the antigens included in ACVs. Clearly such strains are able to cause pertussis, but several lines of evidence suggest that they have a selective advantage over Prn-expressing strains in vaccinated hosts.

For example, vaccinated people appear more likely to be infected by a Prn-deficient strain than one expressing pertactin, as determined by analysis of

cases in the US where there is high coverage with ACVs. [14] Using the mouse model of *B. pertussis* infection it was demonstrated that naïve mice were colonized to the same level by Prn-expressing or Prn-deficient strains. However, in mice immunized with ACV and then subsequently challenged with *B. pertussis*, Prn-expressing strains were cleared more quickly than Prn-deficient ones. [15] Finally, a very recent study developed this further and assessed direct competition between Prn-expressing and Prn-deficient strains in the murine infection model. [16] Coinfection of naïve mice and ACV-immunised mice with equal numbers of a Prn-expressing and a Prn-deficient strain revealed dramatic differences. In naïve mice the Prn-expressing strain became dominant by day 3 post-inoculation and accounted for over 90% of the *B. pertussis* recovered by day 14, demonstrating a distinct fitness advantage from expression of Prn in these hosts. However, in immunized mice, the reverse was observed, as the Prn-deficient strain accounted for over 90% of recovered bacteria by day 14. [16] Together these data very strongly suggest that the loss of expression of Prn generates a fitness advantage to *B. pertussis*, but only in hosts immunized with an ACV. This is compelling evidence to suggest that ACV-induced immunity generates a strong selection pressure on *B. pertussis* and in turn this suggests that this selection pressure has been the driver for the emergence of Prn-deficient *B. pertussis* strains.

That Prn-deficient strains are isolated from people displaying disease symptoms demonstrates that Pertactin is not required by *B. pertussis* for either colonisation of, or pathogenesis in, humans; enabling survival of these strains. If avoidance of immunity is the driver for loss of Prn-expression, then

it might be expected that this would select also for *B. pertussis* strains expressing antigenically variant Prn. Although a number of *prn* alleles have been identified, variation occurs primarily through variability in the number of repeat motifs within the gene, as opposed to mutations altering the amino acid sequence of Prn, discussed in ref. [9] This could be explained by a polyclonal immune response against Prn, targeting multiple regions of the protein. In this case, small changes in protein sequence such as single amino acid substitutions arising from SNPs are unlikely to have much effect on avoidance of this immunity. Data regarding epitopes on *B. pertussis* proteins that are recognised by vaccine-induced immunity is limited. [17] In particular, clear correlates of protection have not been defined and thus it is not possible to identify specific epitopes whose recognition is pivotal for protective immunity that would be expected to exert strong selective pressure. However, current data does support the idea that responses are polyclonal.

This might explain also why the level of variation in vaccine antigen genes, aside from the issue of pertactin-deficiency, among thousands of *B. pertussis* strains analysed, is very low (for example, see refs. [11, 18]) To our knowledge, only 10 *ptxA* alleles, (encoding the PtxA subunit of pertussis toxin, another ACV component) have been described, mostly involving just one or two mutations, and two of which carried synonomous mutations. Thus, there are just 8 protein variants observed in many, many strains isolated world-wide over many decades. In addition, it is clear that a single allele has dominated at any one time. For example, numerous studies since suggest that since 2000, well over 90% of isolates carry the *ptxA1* allele indicating that there has

126 been very little protein variation in PtxA among *B. pertussis* during this time
127 (for example, see refs. [11, 18])
128
129 Interestingly, although few, several PtxA polymorphisms occur in regions that
130 are thought to be B or T cell epitopes [17] but there is no data as to whether any
131 of the polymorphisms result in altered recognition by cells of the immune
132 system.
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134 A similarly very low level of variation is observed for the other ACV antigens
135 (again, for example see refs. [11, 18]). Thus it is not clear why an apparently
136 strong selection pressure on Prn is not apparent for the other ACV antigens.
137 There are key differences between these antigens. Pertussis toxin is a
138 secreted protein complex. Thus, anti-toxin immunity is thought be largely
139 toxin-neutralising rather than targeting the bacterium but the toxin has a key
140 role in *B. pertussis* infection and therefore one would still predict a strong
141 selection pressure to avoid toxin-neutralising immune responses. The
142 expression of fimbrial antigens is subject to phase-variation, via variability in
143 the length of a homopolymeric tract within the *fim* gene promoters. [19] This
144 might provide a mechanism for avoiding anti-Fim immunity in the absence of
145 Fim variation, but if Fim are required for adherence during colonisation and
146 subsequent infection, it is hard to see how anti-Fim immunity does not create
147 selection pressure on these proteins.
148
149 Thus, the role of vaccine antigen variation in *B. pertussis* evolution, or
150 adaptation to vaccine-mediated immunity is not clear at present. However, it is

a very important issue. This is true for considerations about increased use of current ACVs in booster vaccinations in adolescents and adults in response to resurgence. If increased use of vaccines were to further increase the selective pressure for variation, this might increase the rate at which strains emerge that are increasingly mismatched to vaccine-mediated immunity and thus with greater fitness advantages in immunised hosts. The same is true also if ACVs are modified. For example, it has been proposed to 'update' ACVs by including antigens from strains that include antigen variants.

Any development of novel ACVs must also take into account the likely selective pressures that they may create. Genomics has revealed all of the potential *B. pertussis* antigens present in this bacterium. Thus identifying novel antigens is not difficult. Nor is testing their immunogenicity and the protective effect of immune responses against infection, particularly with the availability of an infant baboon model of pertussis. However, just as for current ACVs, avoiding selection pressures that might create short lifespans for novel vaccines is critically important.

Implementing novel *B. pertussis* vaccines, or even modified versions of current ACVs, is a major process to undertake, and likely to be possible only once, or a very limited number of times. Thus it is imperative to understand the effect of antigen variation on immunity in order to inform this process.

In turn, this requires a detailed understanding of the mechanisms of protective immunity against *B. pertussis*, including whether certain epitopes are key to

this in terms of ACV induced immunity. A number of groups are now beginning to identify B and T cell epitopes recognised either during infection or by vaccine-induced immunity (for example, see refs. [20-22], but knowledge regarding protection is still lacking.

In addition, it is important to develop models in which these aspects can be investigated, and resulting hypotheses tested. To this end, the recently developed infant baboon model of pertussis will be vital, and two human challenge models of *B. pertussis* colonisation are in the process of development. While these will be invaluable to answering key questions, they will be limited in terms of the number of studies able to be performed using them, and the number of subjects within each study. The mouse model of *B. pertussis* infection has been much maligned as a tool for investigating resurgence, but it is the one *in vivo* model that can be used widely among research labs and whose ability to use greater numbers of animals than is possible with baboons provides greater power for detecting slight effects. In addition, the availability of genetically modified mice provides powerful tools to investigate the role of specific immune components and processes in immunity to *B. pertussis* and provide *mechanistic* insight. [23]

In summary, the dramatic rise in the incidence of pertactin-deficient *B. pertussis* strains in countries using ACVs, and the demonstration of a fitness advantage of these strains over those expressing pertactin, in immunised hosts, strongly supports the idea that vaccine-mediated immunity creates a selective pressure capable of shaping the genetic make up of *B. pertussis*.

However, this is in contrast to the very low levels of variation observed for the other ACV antigens. Thus, the role of variation among *B. pertussis* in adaptation to vaccination and thus pertussis resurgence is unclear at present but important to determine for understanding resurgence, for expanded use of current ACVs and for the design of novel vaccines to combat *B. pertussis*.

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