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

3

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5

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8

9 *Bordetella pertussis*, resurgence, vaccine, evolution.

10

11 Abstract.

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

21

22 Resurgence.

23 Whooping cough, or pertussis, caused by *Bordetella pertussis* is considered  
24 resurgent in a number of countries despite high levels of vaccination of infants  
25 in these regions. <sup>1</sup> This has involved an increase in the overall number of

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

28

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

46

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

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

53

54 Evolution towards vaccine escape.

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

67

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

73

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

76 cases in the US where there is high coverage with ACVs. <sup>14</sup> Using the mouse  
77 model of *B. pertussis* infection it was demonstrated that naïve mice were  
78 colonized to the same level by Prn-expressing or Prn-deficient strains.  
79 However, in mice immunized with ACV and then subsequently challenged  
80 with *B. pertussis*, Prn-expressing strains were cleared more quickly than Prn-  
81 deficient ones. <sup>15</sup> Finally, a very recent study developed this further and  
82 assessed direct competition between Prn-expressing and Prn-deficient strains  
83 in the murine infection model. <sup>16</sup> Coinfection of naïve mice and ACV-  
84 immunised mice with equal numbers of a Prn-expressing and a Prn-deficient  
85 strain revealed dramatic differences. In naïve mice the Prn-expressing strain  
86 became dominant by day 3 post-inoculation and accounted for over 90% of  
87 the *B. pertussis* recovered by day 14, demonstrating a distinct fitness  
88 advantage from expression of Prn in these hosts. However, in immunized  
89 mice, the reverse was observed, as the Prn-deficient strain accounted for over  
90 90% of recovered bacteria by day 14. <sup>16</sup> Together these data very strongly  
91 suggest that the loss of expression of Prn generates a fitness advantage to *B.*  
92 *pertussis*, but only in hosts immunized with an ACV. This is compelling  
93 evidence to suggest that ACV-induced immunity generates a strong selection  
94 pressure on *B. pertussis* and in turn this suggests that this selection pressure  
95 has been the driver for the emergence of Prn-deficient *B. pertussis* strains.  
96  
97 That Prn-deficient strains are isolated from people displaying disease  
98 symptoms demonstrates that Pertactin is not required by *B. pertussis* for  
99 either colonisation of, or pathogenesis in, humans; enabling survival of these  
100 strains. If avoidance of immunity is the driver for loss of Prn-expression, then

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

115

116 This might explain also why the level of variation in vaccine antigen genes,  
117 aside from the issue of pertactin-deficiency, among thousands of *B. pertussis*  
118 strains analysed, is very low (for example, see refs. <sup>11, 18</sup>) To our knowledge,  
119 only 10 *ptxA* alleles, (encoding the PtxA subunit of pertussis toxin, another  
120 ACV component) have been described, mostly involving just one or two  
121 mutations, and two of which carried synonymous mutations. Thus, there are  
122 just 8 protein variants observed in many, many strains isolated world-wide  
123 over many decades. In addition, it is clear that a single allele has dominated  
124 at any one time. For example, numerous studies since suggest that since  
125 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. <sup>11, 18</sup>)

128

129 Interestingly, although few, several PtxA polymorphisms occur in regions that  
130 are thought to be B or T cell epitopes <sup>17</sup> but there is no data as to whether any  
131 of the polymorphisms result in altered recognition by cells of the immune  
132 system.

133

134 A similarly very low level of variation is observed for the other ACV antigens  
135 (again, for example see refs. <sup>11, 18</sup>). 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 to 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. <sup>19</sup> 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

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

159

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

168

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

173

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



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

180

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

195

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

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

206

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