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What Is Wrong with Pertussis Vaccine Immunity?

Inducing and Recalling Vaccine-Specific Immunity

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The high incidence of pertussis in vaccinated adolescents suggests the failing of immune memory. We argue that acellular pertussis vaccines generate memory cells that are effectively reactivated by boosters better than by *Bordetella pertussis* exposure. We propose that there are two main causes. One is the induction of vaccine-specific immunity rather than pathogen-specific immunity. The second is that strictly mucosal infections such as *B. pertussis* poorly reactivate memory B and T cells residing deep in lymph nodes or tissues. Developing new vaccines for infants or adolescents will be immunologically and economically challenging. Let us hope that maternal and infant immunization, to date the most effective strategies against pertussis death, will remain so.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

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Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

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The resurgence of pertussis is difficult to define and quantitate but it is observed in most countries, which switched to acellular pertussis (aP) vaccines for infant immunization (World Health Organization 2015). Incidence rates vary greatly and are highest in adolescents (Klein et al. 2012, 2016; Acosta et al. 2015; Esposito et al. 2016), although an increase in infant mortality was recently reported for example in the United Kingdom (Amirthalingam et al. 2014; Health Protection Report 2016). The failure of current aP vaccines in adolescents being established, defining its cause would be the obvious way to pave the road toward new vaccines. Yet, because there is an absence of correlates of protection for adolescent pertussis, speculation is the rule.

Many potential epidemiological and immunological contributing factors are being discussed. The widespread availability of polymerase chain reaction (PCR) results in diagnoses being established after only a few days of cough, contrasting with the former use of clinical features including cough for at least 3 weeks and culture confirmation (World Health Organization 1991, 2003). The cyclic outbreak pattern of pertussis epidemics (Fine and Clarkson 1982) and the risks of antigenic shift and/or infection by other strains like *Bordetella parapertussis* or *Bordetella holmesii* (Mattoo and Cherry 2005) confuse the pattern. However, the rapid decay of antibodies and decline of vaccine efficacy in fully primed and recently boosted adolescents (Edelman et al. 2007; Acosta et al. 2015; Esposito et al. 2016; Klein et al. 2016) suggests that the main issue is the waning of immune memory to pertussis. Failures of humoral and cellular responses are being discussed, including the intrinsic role of T cells and/or their failing help to B cells (van Twillert et al. 2015).

We elaborate in this debate our hypothesis that aP vaccine failure already starts at priming. We focus as main causes on the concepts of the original "chemical" sin and the inability to activate mucosal defense and we then discuss future prospects.

FAILURE OF PRIMING BY aP VACCINES

The short-term effectiveness of aP vaccines in aP-primed adolescents correlates with the rapid waning of vaccine-induced antibodies (Le et al. 2004; Lai et al. 2012; Aase et al. 2014). It contrasts with that observed and predicted in whole-cell (wP)-primed adolescents (Bailleux et al. 2008). When antibodies do not persist despite several vaccine doses, it implies that sufficiently strong germinal center reactions (reviewed in De Silva and Klein 2015) have not been generated to elicit high-avidity plasma cells capable of efficiently homing to the bone marrow for decades of survival (Fig. 1A) (Slifka et al. 1998; De Silva and Klein 2015). This implies that the germinal center–derived affinity maturation, selection, differentiation, survival and/or recall of B-cell clones with enhanced antigen affinity have not been successful.

Remarkably, secondary anamnestic antibody responses elicited in children or adolescents appear to not be stronger than primary infant responses (see Table 1; also discussed in Thierry-Carstensen et al. 2013). We found no prospective study directly comparing anti-pertussis toxin (PT) responses of aP-primed subjects following the 4th, 5th, or 6th dose of aP—which prevents evidence-based direct comparisons. However, reported studies that included at least infants and children or children and adolescents (Table 1) did not show any dose-dependent increase nor drastically reduced anti-PT titers with age and successive doses. This suggests that hyporesponsiveness secondary to the loss of memory B cells may not play a critical role, although it may contribute to the picture; in the absence of sufficient boosting (see below), antigen-specific memory may get lost (Posfay-Barbe et al. 2010).

Despite these relatively similar antibody responses, protective efficacy is lower and shorter in adolescents (Witt et al. 2012) than in preschool children (Lambert 2014), suggesting the failure of vaccine memory. Yet, a single dose of aP vaccine effectively reactivates immune memory in individuals primed with wP vaccines (see Huang et al. 2005). Furthermore, a single dose of wP vaccine at priming is sufficient to reduce the risk of adolescent pertussis, and the more wP doses at priming, the better (Sheridan et al. 2012; Baxter et al. 2013; Klein et al. 2013; Witt et al. 2013). Thus, we believe the major problem

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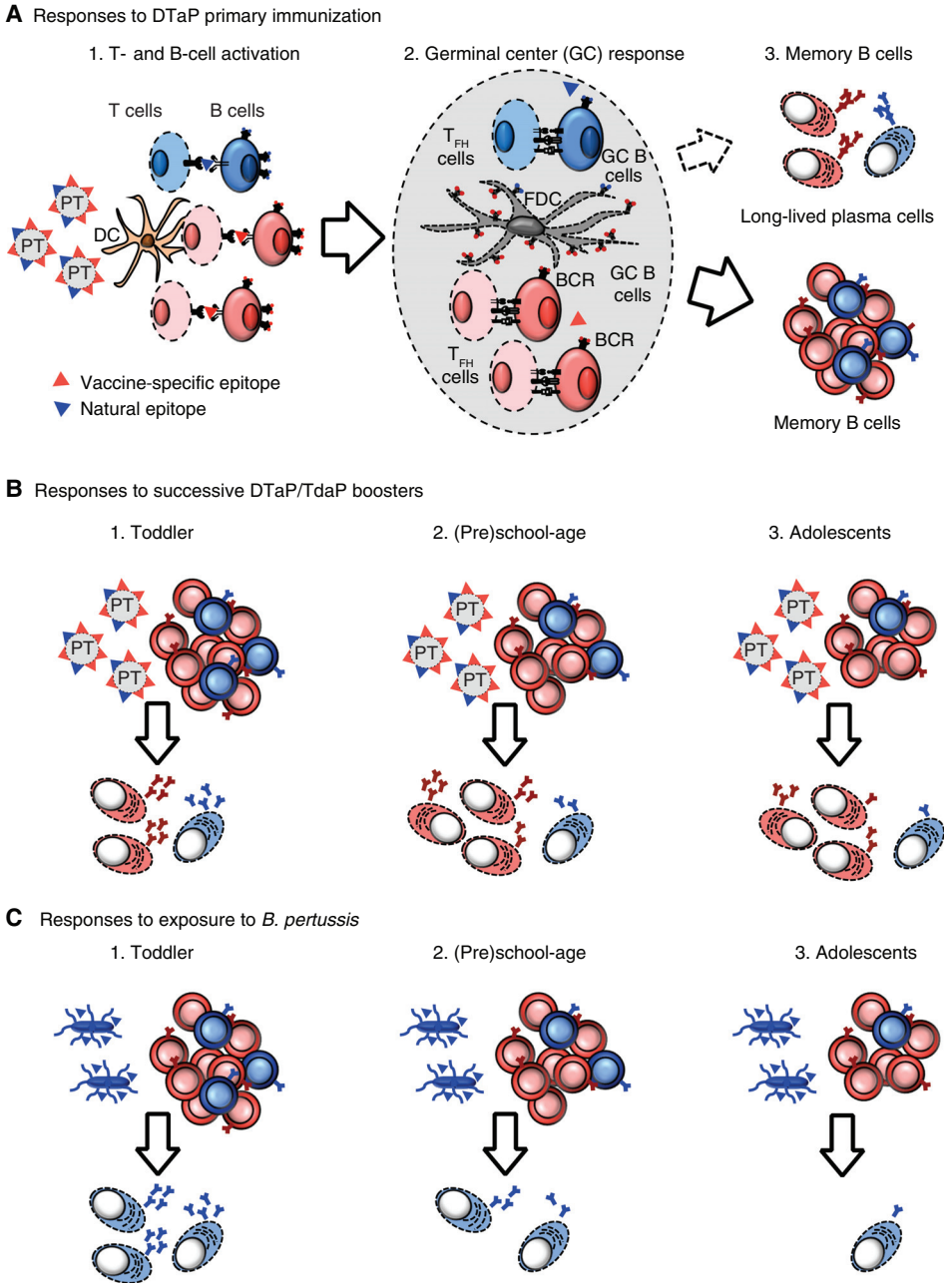

Figure 1. Pertussis toxin B-cell responses to primary and booster acellular pertussis (aP) immunizations or following exposition to *Bordetella pertussis*. Immune response to priming with aP (*A*) leads to formation of anti-pertussis toxin (PT) memory B cells. These are directed either against the epitopes unique to denatured PT (red) or of wild-type PT (blue). Upon boosting (*B*), the proportion of anti-PT memory B cells specific to wild-type PT declines proportionally to the increasing number of doses (and thus age). Consequently, fewer and fewer wild-type PT-specific B cells are available for reactivation at time of exposure to *B. pertussis* (*C*).

Table 1. Comparison of anti-pertussis toxin (PT) antibody concentrations in cohorts of acellular pertussis (aP)-primed children assessed after boosting at various ages

Priming with aP vaccine (PT dose in μg; Nx = doses; age in months)	Age at last aP dose (PT dose in μg)	Anti-PT GMCs (EU/mL ; *N* = number of study subjects)	References
Italy			
DTaP3, 25 μg; 2× (2,4)	6 mo (25 μg)	51.3, CI 95%, 47.9–54.9 (total *N* = 1572)	Greco et al. 1996
DTagdPT3, 5 μg; 2× (2,4)	6 mo (5 μg)	94.4, CI 95%, 88.8–100.3 (total *N* = 1572)	
DTaP3, 25 μg; 3× (2,4,6)	5–6 yr (25 μg)	68.9, CI 95%, 61.00–77.78 (*N* = 277)	Tozzi et al. 2001
DTagdPT3, 5 μg; 3× (2,4,6)	5–6 yr (5 μg)	120.7, CI 95%, 108.32–134.38 (*N* = 281)	
Sweden			
DTaP2, 25 μg; 2× (2,4)	6 mo (25 μg)	Median 50 (estimated from graph) (*N* = 186)	Gustafsson et al. 1996
DTaP5, 10 μg; 2× (2,4)	6 mo (10 μg)	Median 75 (estimated from graph) (*N* = 178)	
DTagdP, 5 μg; 2× (2,4)	6 mo	Median 158 (*N* = 80)	Olin et al. 1997
DTaP5, 20 μg; 2× (2,4)	6 mo	Median 50 (estimated from graph) (*N* = 80)	
DTaP5, 20 μg; 3× (2,4,6)	5 yr (2.5 μg)	22, CI 95%, 20–25 (*N* = 440)	Carlsson et al. 2015
DTaP5, 20 μg; 3× (2,4,6) followed by			
TdaP5, 2.5 μg; 1× (5 yr)	15 yr (2.5 μg)	20, CI 95%, 17–24 (*N* = 114)	Carlsson et al. 2015
	15 yr (20 μg)	74, CI 95%, 61–90 (*N* = 113)	
Germany			
DTaP, 25μg; 2× (3,4)	5 mo (25 μg)	49, CI not reported (*N* = 571)	Schmitt et al. 1996
DTaP, 25μg; 2× (3,4,5)	15–19 mo (25 μg)	109, CI not reported (*N* = 571(?))	Zepp et al. 1996
DTaP, 4× (3,4,5, 15–19 mo)	10–12 yr (8 μg)	51.8, CI 95%, 41.6–64.7 (*N* = 34)	Zepp et al. 2007
TdaP, 1× (5.8 yr earlier)		52.0, CI 95%, 45.3–59.8 (*N* = 93)	
Or DTaP, 1× (5.8 yr earlier)			
Canada			
TdaP5, 2.5 μg, 12–55 yr	>5 yr	144, CI 95%, 132–157 (*N* = 449)	Halperin et al. 2000a
TdaP5-IPV (2.5 μg)	12–18 yr	172, CI 95%, 155–191 (*N* = 350)	Halperin et al. 2000b
Td-IPV, aP alone	Adults	115, CI 95%, 103–128 (*N* = 366)	
	Adolescents	365, CI 95%, 306–434 (*N* = 116)	
TdaP	>10 yr ago (2.5 μg)	116, CI 95%, 105–129 (*N* = 318)	Halperin et al. 2012
Taiwan			
DTaP, 20 μg; 2× (2,4)	6 mo (20 μg)	131, CI 95%, 113–152 (*N* = 64)	Lee et al. 1999
DTaP, 20 μg; 3× (2,4,6)	18 mo (20 μg)	216, CI 95%, 184–253 (*N* = 61)	Lee et al. 1999
DTP, 4×	6–8 yr (8 μg)	89.2, CI 95%, 68.7–115.9 (*N* = 59)	Huang et al. 2005
	15–20 yr (8 μg)	53.3, CI 95%, 43.4–65.4 (*N* = 119)	

Unless stated otherwise, anti-PTantibodies were detected by ELISA 4–12 wk after the booster dose. To minimize confounding factors, we selected a few studies in which children from various age groups were assessed in parallel—as compared to postprimary responses in similar/close cohorts.

is not the waning of memory in adolescence, but the failure of inducing optimal pertussis immunity at time of priming.

The Original "Chemical" Sin

Few antigens are included in current aP vaccines, a key one being PT. PT antibodies are sufficient to protect infants from severe pertussis (Cherry et al. 1998; Storsaeter et al. 1998) and are better markers of vaccine responses as their recall responses are not confounded by exposure to cross-reacting bacteria. In contrast, antibody responses to other antigens such as filamentous hemagglutinin (FHA) can be confounded by cross-reactive bacteria. PT is by nature a moderately immunogenic antigen, which further partly loses its antigenicity when chemically inactivated; it induces low antibody titers even after four doses in infancy and responses to a sixth dose of PT are, remarkably, not stronger than in infants (see Table 1).

That protective efficacy conferred by aP vaccines is lower in immunologically mature, primed, and boosted adolescents than in infants and toddlers is counterintuitive. One potential explanation is that distinct definitions of pertussis are used in various age groups in which awareness, surveillance, and detection systems differ. However, a plausible immunological hypothesis is that of the "original antigenic sin," referred to by Cherry as "linked epitope suppression" (Cherry et al. 2004; Cherry 2013). Initially defined to explain the modest efficacy of repeat influenza vaccines (Kim et al. 2009), this concept implies that the repeated exposure to vaccine-specific antigens progressively generates more and more vaccine-specific effector and memory B cells. These cells increasingly compete with naïve B cells and, thus, the host loses the capacity to adapt and react to the slightly different bacterial epitopes (Cherry et al. 2004, 2010).

During the development of aP vaccines, PT was determined as the main antigen. Its preparation required detoxification for which two options were available in the 1980s: the conventional chemical detoxification of PT after its production or the genetic modification of *Bordetella pertussis* strains producing genetically detoxified PT (Podda et al. 1995; Ibsen 1996). For nonscientific reasons, currently licensed aP vaccines include chemically detoxified PT. Chemical detoxification, however, means protein denaturation and thus potential alterations of the tridimensional structure. A study using competing monoclonal antibodies showed that this denaturation process indeed generates distinct epitopes from the ones present on naïve PT (Sutherland et al. 2011). Thus, infant priming followed by repeated boosting by chemically detoxified PT likely generates effector and memory B cells that are more and more vaccine-specific and less proficient to bind to structurally distinct native PT (Fig. 1B). Differences between natural and vaccine antigens can also arise from a change in the phenotype of pertussis strains (Poolman and Hallander 2007; Bart et al. 2014). However, the showed lack of expression of pertactin (PRN) (Hegerle and Guiso 2014; Lam et al. 2014; Martin et al. 2015) did not affect vaccine effectiveness (Breakwell et al. 2016). We thus see the chemical denaturation of PT, which reduces its modest immunogenicity (e.g., the original "chemical" sin), as mainly responsible for the progressive loss of responses to PT boosters. The direct demonstration of this hypothesis would require substituting chemically for genetically detoxified PT in infant vaccines and following them up to adolescence, a daunting task.

Failure of Postexposure Immune Memory Reactivation

By definition, anamnestic responses reactivate affinity-matured memory B (and T) cells and therefore are faster than primary responses. The contribution of immune memory to protection depends on a number of factors, including the comparative kinetics of immune reactivation and disease; infections with short incubation time, such as *Haemophilus influenzae b* infections, classically occur in primed children (Pichichero 2009). It only takes a few days for antibody titers to increase to significantly higher levels following the boosting of aP-primed children or adolescents (Schure et al. 2013) and pertussis does not follow a rapid course; its average incubation time of 7–10 days should al-

low reactivation of vaccine-induced immunity. Yet, 2–3 weeks are required for anti-PT antibodies to peak in the serum in aP- or wP-primed children or adolescents with PCR-proven pertussis (Hallander et al. 2009)!

Why does pertussis infection fail to rapidly reactivate immunity? *B. pertussis* infection is strictly a mucosal pathogen, with no bacteremia. Therefore, there is likely limited antigen dissemination to the draining lymph nodes where memory B cells essentially reside, expecting to eventually be reactivated by antigen. In addition, *B. pertussis* exerts local immunomodulatory effects, which limit the migration of (antigen-loaded) dendritic cells to the draining lymph nodes (de Gouw et al. 2011; Adkins et al. 2014). Thus, aP vaccines readily reactivate pertussis immunity at time of boosting—but rapid reactivation of pertussis immunity does not occur at time of *B. pertussis* exposure, resulting in infection and symptoms.

Current aP vaccines were designed to induce serum antibodies, and the role of T cells and that of their specific polarization is much discussed (van Twillert et al. 2015). The development of the baboon challenge model (Warfel et al. 2012, 2014) has clearly showed that only wP vaccines reduce the intensity and duration of bacterial colonization (Warfel et al. 2014). Whether this results from the inclusion of too few antigens, the lack of optimized fimbriae (Gorringe and Vaughan 2014), or the preferential induction of Th2 rather than of Th1/Th17 (Warfel and Merkel 2013; Ross et al. 2013) responses by aP vaccines, the limited set of baboon-derived data concurs to the epidemiological evidence. The continued use of wP vaccines in the United Kingdom, providing an average of 15 years of protection contrasting to 5 years for aP vaccines, would have prevented the resurgence of pertussis, and the associated loss of infant lives (Choi et al. 2016).

WHAT LIES AHEAD?

To date, maternal immunization is highly effective against infant death (Amirthalingam et al. 2014; Dabrera et al. 2015), especially if performed in the second trimester (Eberhardt et al. 2016). Beneficial for term infants, second trimester maternal immunization may even contribute to the protection of the most vulnerable preterm infants (Eberhardt et al., in press). As the switch to aP vaccines occurred in the late 1990s or early 2000s, current mothers were primed with wP vaccines. However, the cohort of aP-primed subjects will soon reach childbearing age and be confronted with the same issue of limited protective efficacy as adolescents currently are. It is thus urgent to define whether improved booster vaccines might be developed for future aP-primed pregnant women. Existing options include the use of genetically detoxified PT, shown to be more immunogenic than chemically detoxified PT, inducing higher neutralizing antibody titers and a Th1/Th17 response that may improve mucosal responses (Seubert et al. 2014). To reduce immune escape, additional antigens such as improved fimbriae antigens (Gorringe and Vaughan 2014), adenylate cyclase toxin (ACT), or the autotransporter BrkA could be considered (Brummelman et al. 2015). As the current alum-based adjuvantation does not induce Th1 responses, different toll-like receptor (TLR) agonists are being tested (reviewed in Rumbo and Hozbor 2014) and the use of nanoparticles seems promising in animal models (Gaillard et al. 2014). Finally, nasal live attenuated vaccines inducing dendritic cell (DC) maturation and Th1/Th17 responses are in clinical trials (reviewed in Brummelman et al. 2015).

Will it be sufficient to introduce novel vaccines at the time of adolescent or adult boosting? How many doses of aP vaccines (3, 4, 5, 6, or more?) will be compatible with the effective recall of immune memory by novel adolescent/adult vaccines? These questions are crucial. Indeed, the development of new infant vaccines will be most challenging: first, new vaccines will have to be tested against combination penta- or hexavalent vaccines in current use, requiring very large sample sizes. Next, assessing antibody responses will not be sufficient and infant pertussis efficacy trials will likely be required. This challenging adventure would become logistically and financially impossible should the follow-up period include the demonstration of the

proper induction and reactivation of memory throughout adolescence and adulthood! Should the development of novel booster vaccines fail to properly reactivate aP-primed immunity, the key to the control of pertussis may be to return to its primary objective: to prevent pertussis deaths through maternal and early infant immunization, and to learn again how to live with pertussis outbreaks affecting (and efficiently boosting) older children and adolescents.

CONCLUDING REMARKS

The failure of aP vaccines to induce sufficient immune memory responses and to prevent pertussis in the adolescent is multifactorial and already begins with priming. Improved vaccines that induce potent mucosal defense would be needed. Potential options are to include more antigens, which are more similar to the native ones, to improve adjuvantation or change the route of immunization to improve mucosal defense and reduce colonization. Pending their availability, or should these approaches fail, implementing high levels of maternal and early infant immunization with available pertussis vaccines is crucial and needs to be promoted.

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