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Do we need a new vaccine to control the re-emergence of pertussis?

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Bordetella pertussis **causes whooping cough and is re-emerging in developed countries despite widespread immunization with acellular pertussis vaccines (Pa), which are less effective than the whole cell vaccines that they replaced. Efficacy of Pa could be improved by switching from alum to alternative adjuvants that generate more potent cell mediated immunity.**

The Gram-negative bacterium *Bordetella pertussis* causes whooping cough (pertussis), a severe and prolonged respiratory disease, resulting in substantial morbidity and mortality, especially in infants and young children. There were 16 million cases of pertussis worldwide in 2008 and 195 000 children died from the disease, most from developing countries [1]. However, pertussis is not confined to the developing world, with a recent resurgence of disease in many developed countries, including the USA, Australia, England and Wales, and Ireland (Figure 1). In Australia, the incidence of pertussis has increased from 349 reported cases in 1991 to 38 722 in 2011.

The World Health Organization (WHO) estimated that global vaccination currently prevents 687 000 deaths annually from pertussis [2]. This is clearly a vaccine-preventable disease and vaccine coverage has increased in recent decades, so why are we seeing a resurgence of whooping cough in many developed countries? One explanation may be the switch by most developed countries in the mid-1990s from whole cell pertussis vaccines (Pw) to acellular pertussis vaccines (Pa). When Pw were introduced in the 1940s they significantly reduced the incidence of pertussis, in children, teenagers, and adults. However, the side effects associated with the use of Pw, including a high incidence of fevers and rare cases of convulsions, motivated the development of Pa. Successful Phase III trials in Sweden and Italy in the mid-1990s culminated in the licensing of several Pa. The Pa had substantially improved safety profiles and by the late 1990s most developed countries replaced Pw with Pa, although most developing countries have continued to use Pw.

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The number of pertussis cases continued to decline following introduction of Pa. However, despite high vaccine coverage, better healthcare programs, and strenuous marketing by the vaccine manufacturers, the incidence of pertussis is now increasing in many countries, with dramatic increases in the number of cases in infants, but also a significant proportion of cases in older children and adults [3]. Recent cyclic patterns of pertussis outbreaks every 2–5 years mirror those of the pre-vaccine period, indicating that the circulation of *B. pertussis* in the population has not been disrupted by the vaccination programs. Adults with waning immunity to *B. pertussis* that develop unrecognized coughing illnesses are probably acting as primary reservoirs, transmitting the bacteria to unvaccinated children and non-immune adults. At the recent Tenth International Symposium on Bordetella (Dublin, Ireland, September 2013), the re-emergence of pertussis was a major focus of discussion, with most participants from academia, healthcare professions, and industry agreeing that there was a problem and that we need to re-evaluate Pa and vaccination strategies against *B. pertussis*.

One explanation for the increasing incidence of pertussis in vaccinations is that circulating strains of *B. pertussis* have undergone variation in the antigens included in the vaccine. Immune selection pressure due to vaccination or random mutations in key epitopes of vaccine antigens could allow *B. pertussis* to escape protective immunity induced by Pa. Indeed, there is evidence to suggest that antigenically distinct *B. pertussis* strains have emerged in the past decade with mutations in pertussis toxin (PT) and pertactin causing disease in previously immune or vaccinated hosts [4]. Although antigen variation may allow the development of pertussis in vaccinated individuals, there is no direct evidence that it has caused a complete vaccine failure. Alternatively, it is possible that Pa does not include the combination of *B. pertussis* components required to induce the appropriate antigen-specific immune responses that mediate protection. Virulence factors, such as adenylate cyclase toxin, were not included, yet studies have shown that they can generate protective immune responses in mice [5].

A recent report has demonstrated that protection against pertussis in children vaccinated with Pa is relatively short-lived and wanes substantially each year [3]. This may explain the high incidence of pertussis in 8- to 11-year-old US children who had received the full five doses of Pa in childhood [3] and suggests that Pa have limited ability to induce immunological memory. Finally, it

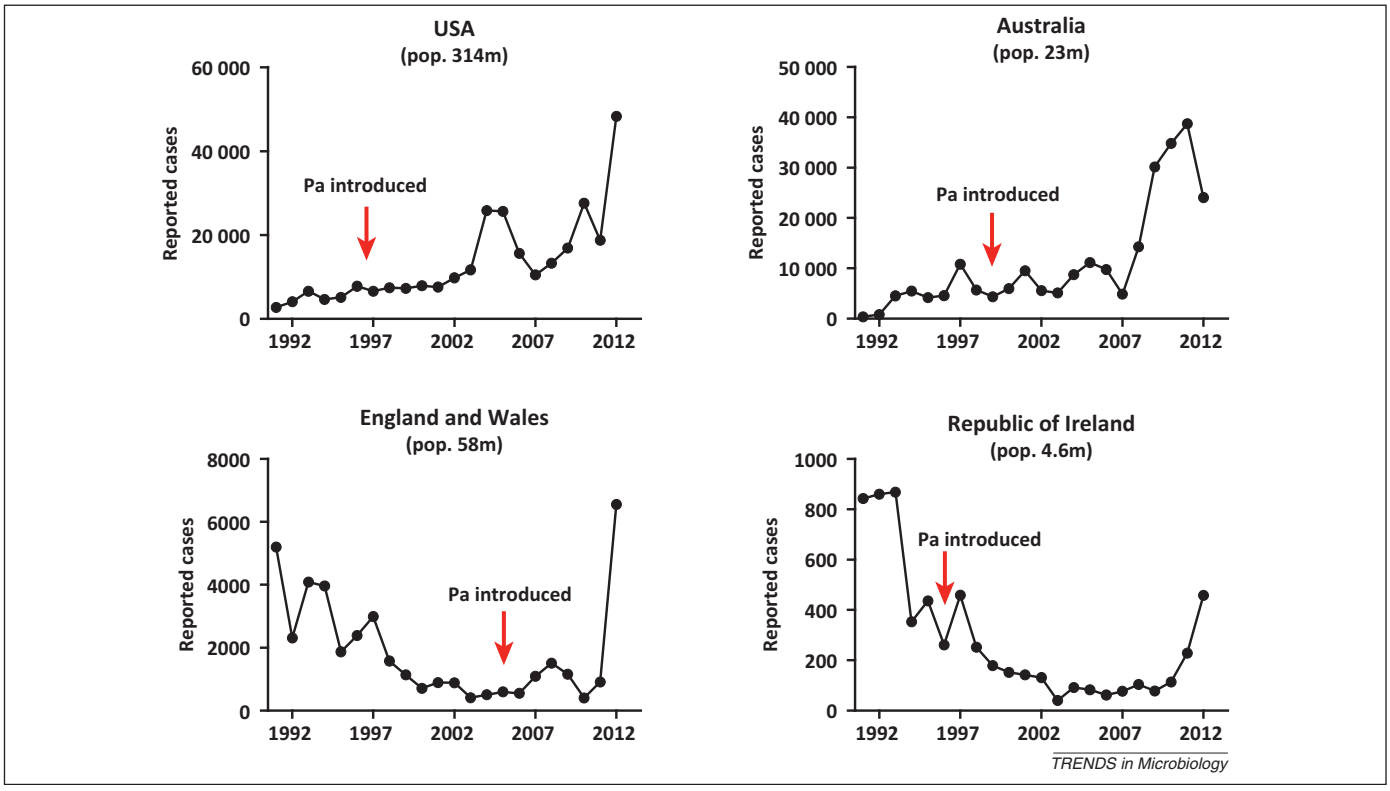

Figure 1. Reported cases of pertussis from 1991 to 2012. Notified cases of pertussis from the USA (data from The Center for Disease Control and Prevention, Atlanta, GA, USA; http://www.cdc.gov/pertussis/fast-facts.html), Australia (data from National Notifiable Diseases Surveillance System, Office of Health Protection, Department of Health and Ageing, Canberra, Australia; http://www9.health.gov.au/cda/source/rpt_2_sel.cfm), England and Wales (data from The Health Protection Agency, London, UK; http://www.hpa.org.uk/hpr/archives/2013/hpr14-1713.pdf), and Ireland (data from The Health Protection Surveillance Centre, Dublin, Ireland; http://www.hpsc.ie/hpsc/A-Z/VaccinePreventable/PertussisWhoopingCough/). The population (pop.) of each country (in 2012) is given in millions in parenthesis after the title. Arrows indicate the introduction of acellular pertussis vaccination programs.

is possible that Pa do not induce the appropriate arm of the immune response that mediates protection.

Advances in our understanding of the mechanism of immunity have been hindered by the view that *B. pertussis* is exclusively an extracellular microorganism and the consequent assumption by many researchers that humoral rather than cell mediated immunity mediates protection. This has motivated a large number of studies assessing antibody responses in children pre- and post-vaccination. Although there are some data pointing to a role for antibodies against PT and pertactin [6], these studies have not provided conclusive evidence that circulating antibodies confer protective immunity in humans and all have failed to find the elusive serological correlate of protection.

Although murine models have been criticized because mice do not develop the same disease or characteristic cough observed in children, the persistent infection with *B. pertussis* has many features of those seen in humans and have proved useful for establishing mechanisms of immunity [5]. Passive immunization studies have shown that *B. pertussis*-specific antiserum can transfer immunity to naive mice; however, protection could also be induced in the absence of antibody and by transfer of T cells alone [7]. Furthermore, studies involving mice defective in key cytokines or cells have provided convincing evidence that CD4 T cells that secrete interferon-γ (IFN-γ; Th1 cells) or interleukin-17 (IL-17; Th17 cells) are critical components of effective adaptive immunity to the bacteria [5]. Complementary studies in mouse models and humans have shown that natural immunity induced by infection or immunization with Pw promotes Th1 responses, whereas Pa induce Th2 and Th17 responses [5] (Figure 2). We believe that this is one of the shortcomings of Pa and reflects the use of alum as the adjuvant.

Alum (aluminum salts) was chosen as the adjuvant for Pa largely because of the ease of regulatory compliance and it had logistical advantages of already being used as an adjuvant in other pediatric combination vaccines. However, data have emerged in recent years suggesting that alum does not direct the appropriate arms of the immune response for optimum protection against *B. pertussis*, and may also fail to induce sustained immunological memory. Studies in animal models have shown that Th1 cells play a critical role in protection against a range of pathogens, including *B. pertussis* [5]. Although there have been claims that alum-adjuvanted Pa induce polarized Th1 responses in children [8], the consensus view is that Pw induce polarized Th1 responses, whereas Pa induce mixed Th1/Th2 or more Th2-skewed responses, especially after frequent booster immunizations in older children [9,10]. The latter is consistent with studies in mice, which have shown that Pa induce polarized Th2 responses, whereas Pw generate Th1 responses that are the critical factors in the high level of protection induced with Pw [11]. Interestingly, recent studies from our laboratory have shown that Pa also induce Th17 responses and that IL-17 and not IL-4, a key Th2 cytokine, is critical for protection with Pa [11]. Collectively, these findings suggest that a