Correspondence

Role of Pertactin in Pertussis Vaccines: The Jury Is Still Out

To the Editor—Hellwig et al. [1] recently described how phagocytic antibodies to pertactin (Prn) play a "crucial role" in *Bordetella pertussis* immunity. Unfortunately, methodological flaws, the relevance of the study results, and unsubstantiated claims undermine their conclusions.

One methodological flaw is that, using unspecified criteria, Hellwig et al. selected serum samples from 16 individuals in a trial of 180 children primed with whole cell–pertussis (wcP) vaccine and boosted at 4 years with wcP vaccine or with 1 of 3 acellular pertussis (acP) vaccines [2]. A second methodological flaw is revealed by comparing table 1 [1] with the results listed in appendix 5 of the original study [2]. This reveals 2 things: (1) that 3 selected donors (donors 7, 9, and 11) were boosted with Takeda acP vaccine (Wyeth Lederle) that contained pertussis toxin, filamentous hemagglutinin, Prn, and fimbriae (Fim), and donor 15 received wcP vaccine, contrary to Hellwig et al.'s claim that all donors received GlaxoSmithKline acP vaccine; and (2) that 4 selected donors (donors 6, 10, 11, and 13) had pre- and postbooster serology suggestive of recent *B. pertussis* or *B. parapertussis* infection (high titers of antibodies to pertussis antigens as well as of IgA). A third methodological flaw is that phagocytosis was evaluated in the absence of exogenous complement; under these conditions, the weak final signal renders the meaning of the inhibition assay questionable. A final methodological flaw is that opsonization with antibodies can promote efficient phagocytosis only in the presence of neutralizing antibodies to adenylate cyclase toxin [3]; but the authors did not measure these.

As for the relevance of Hellwig et al.'s results, there are 3 issues to consider. One is that the contribution of Fc receptors to *B. pertussis* ingestion depends on antibody levels, not just on their presence. Because 75% of donors received an acP vaccine lacking Fim, the relevance of seeking a correlation between phagocytosis and Fim antibody titers is questionable. A second issue is that the roles that Prn and Fim antibodies play were investigated by use of 3 arbitrarily selected serum samples depleted of antibodies: 1 boosted with Takeda vaccine (donor 9) and 2 boosted with GlaxoSmithKline vaccine (donors 13 and 16), including 1 (donor 13) with a probable recent *Bordetella* infection. Such a limited and heterogeneous sample makes conclusions about the role played by Prn antibodies uncertain. Moreover, the assertion that Prn is an important target for antibody-mediated phagocytosis was based on data from only the sample (donor 16) with the highest Prn antibody titer.

A final issue concerning the relevance of the study's results is that pertussis antibodies are known to function in several ways; the promotion of bacterial phagocytosis is only one of them [3]. Immune mechanisms that block bacterial adherence are critical to the early clearance of *B. pertussis* and to the prevention of colonization. Thus, phagocytosis mediated by alveolar macrophages is only a second-line protective mechanism.

In the first of their unsubstantiated claims, Hellwig et al. cite 2 studies of serologic correlates of protection that concluded that arbitrarily defined "high" levels of both Prn *and* Fim antibodies are associated with a lower likelihood of clinical disease, with a weaker correlation for pertussis-toxin antibodies and with no additional benefit from filamentous hemagglutinin antibodies. These data do not support the notion that there is protection by Prn antibodies in the absence of Fim antibodies. Moreover, these 2 studies sought correlations, not causes; did not establish that serum antibodies represent the dominant mechanism of efficacy [4]; and cannot shed light on licensed, efficacious 1- and 2-component vaccines, because the studies failed to include any.

A second unsubstantiated claim is Hellwig et al.'s citation of a secondary source to support their assertion that a study found 3-component acP vaccine significantly more efficacious than 2-component acP vaccine (absolute efficacy of 84% vs. 59%); such a study was never conducted. Quoted efficacy data come from 2 different trials [5, 6], and, as various national advisory bodies have cautioned, each pertussis efficacy trial must stand on its own; results cannot be compared across trials [7, 8]. Furthermore, the 59% efficacy reported for 2-component vaccines applies only to an experimental, unlicensed vaccine. It is inappropriate to use data from a failed vaccine to impugn licensed 2-component acP vaccines with different characteristics, proven efficacy, and established track records. Solid data show that all licensed acP vaccines are highly effective when appropriate coverage rates are achieved, with evidence demonstrating the excellent clinical effectiveness of 1- and 2-component acP vaccines—clinical effectiveness similar to that of multicomponent acP and wcP vaccines—when used in standard clinical practice [9–12; J. Taranger, personal communication].

A final unsubstantiated claim is that, whereas Hellwig et al. assert that Prn variants are causing outbreaks and explain *B. pertussis* persistence, the literature suggests otherwise. First, there is no evidence that increased pertussis in Europe and the United States is linked to *B. pertussis* mutations; rather, surveillance data show

that these increases reflect disease in infants not yet fully immunized and waning vaccine-induced immunity in adolescents and adults. Second, there is no evidence that genotypic variation affects protection against pertussis vaccines; vaccine-efficacy estimates have not decreased in countries with high coverage (e.g., the United Kingdom, France, Finland, and the United States), despite the genetic diversity of *B. pertussis* strains.

In summary, a combination of immune mechanisms provides protection against pertussis; any assumption that Prn is crucial in *B. pertussis* immunity is speculative. Similarly, there is no evidence that the number of antigens in acP vaccines determines efficacy, and there is much evidence to the contrary.

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Reply

To the Editor—Desauziers et al. [1] question the validity of the methodology described in our recent report [2] and state that methodological flaws, unsubstantiated claims, and irrelevance undermine our conclusion that pertactin (Prn)–specific antibodies play an important role in antibody-mediated phagocytosis of *Bordetella pertussis*. We reply to these criticisms as follows.

Desauziers et al. point out that an error was made in the description of serum samples in table 1. We acknowledge this mistake (see the Erratum in this issue of the *Journal*). It is important to note that this mistake does not alter the results described in our study, nor does it undermine our conclusions. The serum samples that we used were selected because they displayed significant titers of antibodies to multiple *B. pertussis* antigens, so that we could study the opsonic quality of specific antibodies.

In addition, Desauziers et al. question the validity of our methodology. They claim that phagocytosis can be promoted only in the presence of neutralizing antibodies to adenylate cyclase toxin and of complement. Unfortunately, Desauziers et al. refer to studies in which our methodology was not used [3] and do not present new data that contradict our results. Furthermore, Desauziers et al. ignore our previously published studies, in which our methodology is described and validated in detail. In brief, these studies showed that immune serum is capable of promoting phagocytosis of *B. pertussis* in the absence of complement [4]. Using flow cytometry–based methodology, one of us found no indications that phagocytosis of *B. pertussis* is promoted only in the presence of adenylate cyclase toxin antibodies (M.E.R., unpublished data).

In an attempt to argue that our conclusion—that phagocytosis is largely induced by Prn antibodies—is "unsubstantiated," Desauziers et al. refer to a recent study by Mobberley-Schuman et al. [3]. In that study, fluorescence microscopy was used in an effort to show that phagocytosis of *B. pertussis* depends on the presence of neutralizing antibodies to adenylate cyclase toxin. We had previously discussed the possible drawbacks of microscopy for phagocytosis experiments [4]; we regret that Desauziers et al. chose not to refer to that earlier study.

Desauziers et al. question the relevance of seeking a correlation between phagocytosis and the presence of fimbraie (Fim)–specific antibodies because most donors received an acellular pertussis (acP) vaccine lacking Fim antibodies. However, the primary inclusion criterion for serum