Distinct T-cell subtypes induced with whole cell and acellular pertussis vaccines in children

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SUMMARY
Recent clinical trials have demonstrated that new generation acellular pertussis vaccines can confer protection against whooping cough. However, the mechanism of protective immunity against Bordetella pertussis infection induced by vaccination remains to be defined. We have examined cellular immune responses in children immunized with a range of acellular and whole cell pertussis vaccines. Immunization of children with a potent whole-cell vaccine induced B. pertussis-specific T cells that secreted interferon-γ (IFN-γ), but not interleukin-5 (IL-5). In contrast, T cells from children immunized with acellular pertussis vaccines secreted IFN-γ and/or IL-5 following stimulation with B. pertussis antigens in vitro. These observations suggest that protective immunity conferred by whole-cell vaccines, like natural immunity, is mediated by type 1 T cells, whereas the mechanism of immune protection generated with acellular vaccines may be more heterogenous, involving T cells that secreted type 1 and type 2 cytokines.

INTRODUCTION
The traditional vaccine against whooping cough composed of whole killed Bordetella pertussis bacterial cells has been successful in controlling pertussis in most developed countries.1,2 However, concerns over its reactogenicity have motivated the development of safe and effective acellular vaccines manufactured using highly purified antigens of B. pertussis. A range of acellular pertussis vaccines, comprising one or more of the antigens, detoxified pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN) and fimbriae (FIM), have recently been evaluated in phase 3 clinical trials in Sweden, Italy, Germany and Senegal.3-8 When compared with the whole-cell vaccines, the incidence of adverse events associated with immunization of children with the acellular vaccines was considerably reduced.3-9 However, the estimated efficacy of the acellular vaccines did not rival that of most European whole-cell vaccines.2,3-7 Furthermore, it is not clear that the persistence of the immune response elicited with acellular vaccines will approach that generated by respiratory infection or immunization with the whole-cell vaccine and the mechanism(s) of protective immunity induced by pertussis vaccination is still poorly characterized.

Apart from preliminary evidence from a household contact study,10 the clinical trials have failed to show a clear correlation between levels of serum antibodies to B. pertussis antigens and protection.3-9,11 While circulating antibodies are known to play a role in both toxin neutralization and prevention of bacterial attachment to respiratory epithelial cells, evidence is emerging that humoral immunity alone may not be sufficient to confer protection against B. pertussis infection.12-14 It is now accepted that B. pertussis is not exclusively an extracellular pathogen, but that it has the ability to invade and survive within mammalian cells, including alveolar macrophages,15,16 suggesting that cell-mediated immunity may be required to completely eliminate invasive bacteria. Studies in a murine respiratory infection model have demonstrated that the adoptive transfer of CD4+ T cells from immune mice can confer protection to nude or immunosuppressed recipient mice against aerosol challenge with live B. pertussis in the absence of a detectable serum antibody response.13 These protective T cells
were shown to secrete high levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ), but not IL-4 or IL-5, a cytokine profile characteristic of T helper 1 (Th1) cells.\textsuperscript{13,14,17} Furthermore, immunization of mice with a whole cell pertussis vaccine generated Th1 responses and moderate antibody levels\textsuperscript{14,17,18} and conferred a high level of protection against respiratory infection.\textsuperscript{14} In contrast, an acellular vaccine induced Th2/Th0 cells and strong antibody response, and did not confer the same level of protection in the murine respiratory challenge model.\textsuperscript{14,17}

We have already reported that \textit{B. pertussis} infection in children is associated with preferential activation of type 1 Th cells.\textsuperscript{19} As recovery from a non-lethal \textit{B. pertussis} infection confers immunity that provides long-lasting protection against subsequent disease, in children as well as in mice, this suggests that cellular responses mediated by Th1 cells may play a key role in protective immunity against \textit{B. pertussis}. Although whole-cell and acellular pertussis vaccines have also been shown to stimulate \textit{B. pertussis}-specific T-cell proliferation in humans,\textsuperscript{20–23} the nature of these responses are only beginning to be addressed.\textsuperscript{24–26} In this study we have examined the production of T-cell cytokines following \textit{in vitro} antigen stimulation of peripheral blood mononuclear cells (PBMC) from children immunized with whole-cell vaccines or with a range of new acellular vaccines. We demonstrate that whole-cell pertussis vaccines selectively induces the activation of \textit{B. pertussis}-specific type 1 T cells, whereas acellular pertussis vaccines induce T cells with a more heterogenous cytokine profile.

\section*{MATERIALS AND METHODS}

\subsection*{Subjects and vaccination schedules}

Fresh peripheral blood was obtained from British, Swedish and Norwegian children immunized with either the Wellcome/ Medeva (W/M) or Connaught Laboratories Inc., USA (CLI) whole-cell pertussis vaccine (Pw) or the SmithKline Beecham (SB) 3-component (detoxified PT, FHA and PRN) SB 2-component (detoxified PT and FHA), Chiron Biocine (CB) 3-component (recombinant PT, FHA, PRN), Connaught Laboratories Ltd, Canada (CLL) 5-component (detoxified PT, FHA, PRN and FIM 2+3) or Pasteur Mérieux (PM) 2-component (detoxified PT and FHA) acellular vaccines (Pa). Informed consent was obtained from the parents of all children used as donors of blood samples in this study. Ethics committees of the Medical Faculties of Umeå University Hospital, Linköping University, University Hospital Bergen, the North and East Hertfordshire District and the John Radcliffe Hospital approved the studies. The details of the study populations and blood sampling schedules are summarized in Table 1. The study of Swedish children were initiated after the commencement of the pertussis clinical trials (1992–95 and 1993–96), therefore it was not possible to obtain fresh blood samples from the same children before and after immunization. However, control blood samples were obtained from naive Swedish children of a similar age who had not been vaccinated against pertussis and had not suffered from clinical pertussis.

\subsection*{Bacterial antigens}

Heat-killed \textit{B. pertussis} W28 was prepared as previously described.\textsuperscript{13} Purified PT, FHA and PRN were provided by the manufacturers: SmithKline Beecham, Rixensart, Belgium, Connaught Laboratories Ltd, Ontario, Canada and Chiron Biocine Institute of Immunological Research, Siena, Italy. PT was inactivated by heating at 80° for 20 min. All other antigens were used as native preparations without chemical or heat inactivation.

\section*{T-cell proliferation and cytokine assays}

Fresh venous blood samples were taken into preservative-free heparinized tubes and sent by courier to Ireland, where they were processed within 24 hr. Blood was diluted 1:2 with RPMI-1640 medium and layered on a Ficoll gradient and centrifuged at 400 g for 30 min. PBMC were removed from the gradient, washed twice and resuspended in RPMI-1640 medium supplemented with 8% fetal calf serum. PBMC were cultured in triplicate in 200 μl wells of 96-well microtitre plates at 1–1.5 × 10⁶/ml with heat-killed bacteria (10⁶–10⁷ bacteria/ml), FHA (1–10 μg/ml), heat inactivated PT (0.1–5.0 μg/ml), PRN (1–10 μg/ml) for 6 days at 37° in a humidified CO₂ incubator. Control stimuli included medium alone or anti-CD3 (OKT3, 2 μg/ml) and phorbol 12-myristate 13-acetate (PMA) (25 ng/ml). Because of limitations on cell yield from the blood volumes available, not all PBMC preparations were tested against the full range of antigens. Proliferation was assessed by [³H]thymidine incorporation after 6 days of culture, as described.\textsuperscript{19}

Cytokine analysis was performed on supernatants of PBMC stimulated as described for the proliferation assay. Supernatants were removed after 72 hr and the levels of IFN-γ, and IL-5 measured by immunoassays, as described.\textsuperscript{19} The limits of sensitivity of the assays were 0.25 U/ml for IFN-γ and 15 pg/ml for IL-5. In certain experiments the concentrations of IL-2 were also determined using the cytotoxic T lymphocyte line (CTLL) bioassay\textsuperscript{13} on supernatants removed after 24 hr.

\subsection*{Flow cytometry analysis of cultured cells}

Surviving cells were recovered from antigen-stimulated PBMC cultures following the removal of supernatants for cytokine analysis. Cells were washed twice and then labelled with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)- conjugated anti-human monoclonal antibodies specific for CD3, CD4 or CD8 (Becton Dickinson, San Jose, CA). Flow cytometry was performed on a Becton Dickinson FACScan.

\section*{RESULTS}

\subsection*{Validation of assays and response in naïve children}

Because all of the children in this study were not evaluated at the same interval after immunization, we have not attempted to make direct quantitative comparisons between different vaccines. Our primary objective was to look at the pattern of type 1 and type 2 cytokines produced by PBMC from children immunized with whole cell and acellular pertussis vaccines. Analysis of T-cell responses in un-vaccinated children enabled the discrimination between \textit{B. pertussis} antigen-specific responses induced by vaccination compared with that present in a ‘naïve’ population prior to vaccination. As we have previously reported,\textsuperscript{19} PBMC from a proportion of unvaccinated children did display positive \textit{B. pertussis}-specific T-cell responses, but this was restricted to IFN-γ production (Figs 1–3). IFN-γ levels greater than 0.5 U/ml were detected in
culture supernatants of PBMC from 29% of children examined in response to at least two of the *B. pertussis* antigens: PT, FHA and PRN. Polyclonal activation with anti-CD3 and PMA revealed that PBMC from all naive children examined were capable of secreting high levels of IFN-γ (≥ 50 U/ml) and IL-5 (≥ 1000 pg/ml) (data not shown). IL-5 was chosen as a marker of Th2 activation as IL-4 is difficult to measure in culture supernatants, especially with PBMC from children.

**Selective induction of type 1 T cells with whole cell pertussis vaccines**

Twenty-five 12–13-month-old children that had received three doses of the W/M Pw vaccine at 2, 3 and 4 months, as part of a clinical trial in Oxford, UK, were assessed 8–9 months after vaccination. Four 1–2-year-old children that had received primary vaccination with three doses of the W/M Pw at 3, 5 and 10 months as part of the routine immunization programme in Norway were tested 2–8 months post vaccination. Moderate to high levels of IFN-γ was detected in supernatants of PBMC following *in vitro* stimulation with PT (66% samples tested), FHA (62%) and PRN (72%) (Figs 1–3). Furthermore high levels of antigen-specific IFN-γ production was detected in 78% of children following stimulation of PBMC with killed *B. pertussis*, but not with medium only (data not shown). Positive IFN-γ responses to at least two of the *B. pertussis* antigens was detected in 90% of the vaccinated children examined. In contrast, IL-5 was undetectable following stimulation of the same PBMC preparations with any of the *B. pertussis* antigen preparations tested (Figs 1–3). Stimulation with anti-CD3 and PMA revealed that these PBMC were capable of producing IL-5 (100–→ 1000 pg/ml) following polyclonal activation. Therefore it appears that vaccination with the W/M Pw preferentially activates *B. pertussis*-specific type 1 T cells. Assessment of the T-cell responses in children recruited for a pre-school booster study in Oxford demonstrated IFN-γ production in response to killed *B. pertussis* in PBMC from 4-year-old children who had received a full immunization schedule with the W/M Pw in the first year of life (Fig. 4b). Although the responses were not as strong as those in recently vaccinated children, they do demonstrate that T-cell responses persist for at least 4 years after immunization with a whole-cell vaccine. Nineteen children that had been immunized at 2, 4 and 6 months with CLI Pw in the Stockholm efficacy trial 1992–95 were assessed 2–4 years after immunization. IFN-γ levels greater than twice the sensitivity of the assay were detected in PBMC supernatants from 36, 21 and 6% of children in following *in vitro* stimulation with PT, FHA and PRN, respectively. Positive T-cell responses against at least two antigens were only detected in 16% of children examined. Furthermore, *B. pertussis*-specific IL-5 production was only detected in one PBMC sample (Fig. 2). The data suggests that the CLI Pw, which had only shown 36/48% efficacy in the Italian/Swedish trials, is poorly immunogenic for T cells, but that any T cells induced have a type 1 cytokine profile.

**Acellular pertussis vaccines induce T cells that secrete type 1 and type 2 cytokines**

Blood samples were obtained from 33 children that had been randomized to the placebo arm in the Stockholm efficacy trial 1992–95 and had received two to three immunizations with either the SB Pa3, CB Pa3 or CLL Pa5 in follow up studies at 3–4 years of age. Immune responses were also assessed in an additional 15 children 2–4 years after immunization.
Figure 1. IFN-γ and IL-5 production by PT-specific T cells following immunization of children with whole cell or acellular pertussis vaccines. Children had been immunized with either the W/M Pw (squares, UK children examined 8–9 months after immunization; triangles, Norwegian children examined 2–8 months after immunization); CLI Pw; SB Pa2 (squares, children examined 2–4 years after immunization; triangles, children examined 8–14 months after immunization); SB Pa3, CLL Pa5 (squares, children examined 2–4 years after immunization; triangles, children examined 1–4 months after immunization) CB Pa3, or were not vaccinated against pertussis. Results are presented for individual children as mean IFN-γ and IL-5 production in supernatants of triplicate PBMC cultures in responses to the optimum concentration of PT. The responses are arbitrarily designated as Th2, Th1/Th2, undetectable and Th1, if they fall within the four marked quadrants (read left to right top to bottom); the numbers in each quadrant represent the number of samples with values within that quadrant.
**T-cell subtypes in vaccine-induced immunity to B. pertussis**

![Graphs showing IFN-γ and IL-5 production](image)

Figure 2. IFN-γ and IL-5 production by FHA-specific T cells following immunization with whole cell or acellular pertussis vaccines. Experimental details as described in Fig. 1.

PBMC from 40% of children immunized with SB Pa3 secreted significant levels of IFN-γ and IL-5 indicative of a Th0 or a mixed Th1/Th2 response in response to PT, FHA and PRN. PBMC from the remainder of children immunized with this vaccine produced IFN-γ only (PT, 40%; FHA, 10%; PRN 10%) or IL-5 only in response to PRN (20%) (Figs 1–3). A positive T-cell response to at least two of the purified antigens was detected in 60% of the children examined. Although not routinely tested on all blood samples (because of limited cell yield from small blood volumes available), antigen-specific proliferation and IL-2 production was also assessed in eight children immunized with the SB Pa3 and in three naive children on the same days. PBMC from children immunized with the SB Pa3 proliferated and secreted IL-2 when stimulated *in vitro* with inactivated PT, FHA, PRN and killed *B. pertussis* (Fig. 5). Although proliferation and low levels of IL-2 was detected in response to killed *B. pertussis* in one of the naive children, the response to the purified antigens were significantly greater in the immunized children (Fig. 5).
The CLL Pa5 also induced T cells with a heterogenous cytokine profile. IFN-γ and IL-5 production was detected in antigen-stimulated PBMC from 17, 29 and 29% of children examined in response to PT, FHA and PRN, respectively. Th1-like response, with the production of IFN-γ, but not IL-5, were detected in 33, 29 and 17% of vaccinees in response to PT, FHA and PRN, respectively. Th2-like responses (IL-5 only) were detected in 4% and 12% of vaccinees following in vitro stimulation of PBMC with PT and PRN, but none with FHA (Figs 1–3). Positive T-cell responses to at least two of the B. pertussis antigens were detected in 54% of vaccinated children. Similar patterns of cytokine secretion were detected in antigen-stimulated PBMC from recently vaccinated children and children immunized 2–4 years earlier.

Although the numbers of children examined were too small to detect statistically significant differences, the CB Pa3 prepared with genetically detoxified PT combined with FHA and PRN, appeared to induce T-cell responses which were more polarized to the Th1 subtype. Positive IFN-γ responses were detected in 71% of vaccinated children, whereas a Th2 cytokine pattern (IL-5 only) was not detected in response to any of the antigens in any of the PBMC preparations examined. PBMC from 43, 57 and 43% and vaccines secreted IFN-γ, but undetectable IL-5 following in vitro stimulation with PT, FHA and PRN, respectively. A mixed Th1/Th2 cytokine profile characterized by the detection of significant levels of IFN-γ and IL-5 was observed in 7, 7 and 21% of vaccinees in responses to PT, FHA and PRN, respectively (Figs 1–3). Positive T-cell responses to at least two of the B. pertussis antigens were detected in 64% of vaccinated children.

Late post vaccination blood samples were also obtained from 31 children 2–4 years after immunization with three doses of SB Pa2 (Stockholm efficacy trial 1992–95) and an additional 12 children (1–2 years old) that had been immunized with the SB Pa2 (Stockholm efficacy trial 1993–96) were assessed 8–12 months after vaccination. The main feature of the T-cell response in children immunized with the SB Pa2 was the low or undetectable levels of IL-5. Low but significant levels (greater than twice the sensitivity of the assay) of IFN-γ were detected in 50 and 54% of PBMC preparations in response to PT and FHA and an additional 12% also secreting IL-5 following stimulation with PT (Figs 1–2). However, the levels of IL-5 were below the limit of detection in the supernatants.
of all PBMC preparations examined following stimulation with FHA. Interestingly, the positive IL-5 production was only detected in recently vaccinated children, whereas IFN-γ responses were stronger in children tested at more prolonged intervals after immunization. Therefore it is possible that a proportion of the Th1 responses detected were not induced by immunization, but were primed by subclinical infections with B. pertussis. Positive T-cell responses to at least two of the B. pertussis antigens were detected in only 38% of vaccinated children, reflecting the poor immunogenicity of this vaccine.

**Effect of booster immunization**

Pre- and post-booster immune responses were assessed in 10 children who had received a full course of immunization with the SB Pa2 at 2, 4 and 6 months of age and boosted 4 years later with the SB Pa3. In comparison with the pre-booster responses, significantly higher levels of IL-5 were detected in antigen-stimulated PBMC supernatants following booster vaccination (Fig. 4a).

In a second study, 36 children who had received W/M Pw as part of the routine diphtheria tetanus and pertussis (DTP) vaccination at 2, 3 and 4 months were examined 1 month after boosting with DT only or DTPa (CB Pa3 or PM Pa2) at 4 years of age. PBMC from the children which received DT vaccine only had detectable levels of IFN-γ, but no IL-5, when stimulated in vitro with killed B. pertussis (Fig. 4b). In contrast, PBMC from a proportion of the children that received the booster dose with the acellular vaccines produced IFN-γ and low levels of IL-5 in response to purified B. pertussis antigens. By comparison with the study shown in Fig. 4a the cytokine profile was more polarized to Th1 than to Th2 type T cells, suggesting that the primary course of immunization may have an impact on the immune response generated after booster immunization.

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Figure 5. Induction of proliferation and IL-2 producing T cells in response to killed *B. pertussis* and purified antigens following immunization with the SB Pa3. Eight 2-4-year-old children were examined 2-4 months after a full course of vaccination; three un-vaccinated children (5*, 10*, and 11*) were tested on the same day. Results are expressed as mean [3H]thymidine incorporation or units of IL-2 for triplicate cultures of PBMC from individual children in response to killed *B. pertussis*, inactivated PT, FHA and PRN. The filled portion of the bars represent the background response to medium alone.

**Phenotype of cytokine producing cells**

The practical and ethical consideration of taking large blood samples from healthy infants precluded the use of purified CD4+ T cells and autologous antigen presenting cells for assessment of T-cell cytokine production. However, we attempted to identify the main source of the cytokines detected by phenotypic analysis of the antigen-stimulated PBMC. Flow cytometry analysis of antigen-stimulated PBMC recovered from cultures following removal of supernatant for cytokine analysis revealed that 81.5±3.9% of the surviving cells were T cells and of these 76.6±8.3% were CD4+ and 22.8±7.5% CD8+ (mean±SD values from 13 immunized children), suggesting that responding cells were predominantly CD4+ T cells.

**DISCUSSION**

The findings of this study provide the first evidence that whole cell and acellular pertussis vaccines activate different subpopulations of T cells in immunized children and suggest that these vaccines may confer protection against whooping cough using distinct combinations of cellular and humoral immunity. Although clinical trials have demonstrated that new generation acellular pertussis vaccines can confer a high level of protection against World Health Organization (WHO)-defined whooping cough, with fewer side effects than the traditional whole-cell vaccines, an immunological correlate of protection still remains elusive. Despite extensive investigations on the induction of antibody response with pertussis vaccines, there is increasing evidence, especially from murine models, that T cells
may also play a fundamental role in protection against infection with *B. pertussis*. However, evaluation of cell-mediated immunity has been largely overlooked in the study design of most of the vaccine efficacy trials.

We have already reported potent *B. pertussis-*specific Th1 responses during and after recovery from whooping cough and a number of reports have described the induction of *B. pertussis-*specific T-cell responses in adults and more recently in children immunized with acellular or whole cell pertussis vaccines, using proliferation as an index of T-cell activation. Cassone et al. studied T-cell proliferative responses in children immunized with the CL1 Pw, SB Pa3 or CB Pa3 and concluded that acellular pertussis vaccines are better inducers of cell-mediated immunity than whole cell vaccines. In contrast, the results of our study based on cytokine production, demonstrate that high efficacy whole-cell (W/M Pw) and acellular (SB Pa3, CLl Pa5 and CB Pa3) pertussis vaccines both induce detectable T-cell responses in a high proportion of immunized children, but may prime distinct T-cell subsets in vivo. In the present study we have demonstrated that immunization with a whole-cell vaccine, like natural infection, selectively induces the activation of pertussis-specific T cells that secrete high levels of IFN-γ but not IL-5, a profile characteristic of the Th1 cells. In contrast immunization with acellular pertussis vaccines induces a mixed Th1/Th2 or Th0 response.

Zepp et al. have also reported that immunization of infants with the SB Pa3 induced IL-2- and IFN-γ-producing Th1 cells. Using the same vaccine in a different study population, we also demonstrated antigen-specific proliferation, IL-2 and IFN-γ production, but in addition detected significant levels of IL-5. Although our conclusion of a mixed Th1/Th2 profile differs from that of a preferential induction of Th1 cells proposed by Zepp and colleagues, the data are not necessarily incompatible. Zepp et al. used IL-10 as an index of Th2 cells and did not measure IL-5 or other Th2 cytokines. However, IL-10 is not produced by all Th2 clones and its secretion is not confined to Th2 cells, or even to T cells, because macrophages are also important producers of this cytokine. The different immunization schedule and age of the children in the two studies may also have influenced the detection of Th cell subsets. In our study the children received two or three immunizations with the SB Pa3 at 4 years of age, whereas in the study by Zepp et al. the children were immunized at 3, 4, 5 and 15–19 months. However, our demonstration that the age of the children did not affect the Th1 or Th2/Th1 cytokine profiles detected in children immunized with the W/M Pw or CLl Pa5, respectively, suggests that the age difference is unlikely to have affected the cytokine profiles observed.

It might be argued that the detection of modest levels of Th1, but not Th2 cytokines, with the SB Pa2, compared with a mixed Th1/Th2 cytokine profile with SB Pa3, suggests that the lower efficacy of the SB Pa2 may reflect a failure to induce Th2 cells. However, the IFN-γ production detected in PBMC from children immunized with the SB Pa2 was primarily detected in children examined 2–4 years after vaccination. During the same period a proportion of unvaccinated children in Sweden also appeared to acquire Th1 responses against *B. pertussis* antigens. Therefore, it is plausible that during the 2–4-year period between immunization and testing, these responses were induced by subclinical pertussis, which was very common in Sweden in this period and which we have previously shown to be associated with selective induction of Th1 cells. Formal proof this hypothesis will only be possible following analysis of T-cell responses induced with acellular pertussis vaccines in countries with very low incidence of pertussis. The observation that T-cell responses are maintained in children immunized with the CL1 Pa5 and W/M Pw suggests that the poorer efficacy of the SB Pa2 and CL1 Pw reflects generalized poor immunogenicity of these preparations. However, this conclusion does not rule out a protective role for PRN in acellular vaccines, as this component was a consistent inducer of antibody (Ryan et al. unpublished observations) and Th2 responses, especially with the SB Pa3.

The dogma that Th1 and Th2 cells are associated with cell-mediated and humoral immunity, respectively, has recently been re-evaluated. Our studies with the murine *B. pertussis* respiratory model and a transgenic model for poliovirus have demonstrated that specific subclasses of immunoglobulin G (IgG) stimulated by Th1 cells can play a significant role in protection against intracellular pathogens. Therefore, if antibodies do play a role in vaccine-induced immunity against *B. pertussis*, either Th1 or Th2 cells may be involved in the provision of T-cell help. In contrast, IFN-γ, secreted by Th1 cells appears to be essential for activating killing of intracellular *B. pertussis* (Mahon and Mills, unpublished observations). The polarized Th1 response induced by infection or with high efficacy whole-cell vaccines [present study], suggests that priming of this arm of the immune response may be essential for complete protection against *B. pertussis*. Although IFN-γ was produced by PBMC from the majority children immunized with the high efficacy acellular vaccines, in response to one or more of the *B. pertussis* antigens, a significant proportion of the samples also secreted Th2 type cytokines. Furthermore, production of Th2 cytokines was significantly enhanced in children recently boosted with acellular vaccines.

The dichotomy of T-cell induction with the two forms of the pertussis vaccine are consistent with the hypothesis that a particulate immunogen containing low levels of toxins that stimulate IL-12 and IFN-γ production will result in selective induction of Th1 cells, whereas highly purified alum-adsorbed soluble *B. pertussis* antigens devoid of toxin activity, will result in the induction of both Th1 and Th2 subtypes of T cells. We have recently demonstrated that the presence of lipopolysaccharide (LPS), which stimulates IL-12 production, and active PT which has adjuvant properties for T cells, although major contributors to reactogenicity of the whole cell vaccine, may also make a positive contribution to their protective efficacy. Furthermore, the addition of IL-12 to an acellular vaccine formulation significantly enhances its potency by stimulating the induction of Th1 cells.

When taken together with our findings from the mouse model, the results from the present study suggest that the idea of defining a single immune correlate of protection may be an over simplistic and unattainable goal. It appears that the mechanism of protection involves a complex combination of antibody and T-cell responses against multiple antigenic components of *B. pertussis*. Our findings suggest that Th1 cells mediate protection induced by whole-cell vaccines and that the mechanism may involve activation of the antimicrobial activity of macrophages, as well as help for specific subclasses of IgG involved in opsonization. In contrast, antibody responses may have a greater role in immunity generated with acellular vaccines and here distinct subclasses of IgG may
confer protection by inhibition of bacterial adherence to ciliated epithelial cells and neutralization of toxins. However, the dramatic fall in serum antibody levels as early as 14 months after vaccination with theacellular pertussis vaccines, suggest that persistent cellular responses induced with acellular and whole-cell pertussis vaccines may play a significant role in long-term protection against B. pertussis.

ACKNOWLEDGMENTS
This work was supported by the Irish Health Research Board (project grant number 100/94) and the National Institute of Health, USA (grant number NO1AI115125). We thank the children, parents and nurses for their cooperation and help with these studies.

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