Immunogenicity and protective efficacy of neonatal vaccination against Bordetella pertussis in a murine model: evidence for early control of pertussis

RODUIT, Caroline Monique, et al.

Abstract

A significant resurgence of early cases of pertussis is being observed in infants too young to have yet completed their three-dose vaccination schedule. In this study, murine models of immunization and Bordetella pertussis challenge were adapted to early life. This allowed comparative evaluation of immunogenicity and protective efficacy of immunization initiated in the neonatal period (7-day-old mice) or in infancy (3-week-old mice) with diphtheria-tetanus-whole-cell pertussis (DTPw) and diphtheria-tetanus-acellular pertussis (DTPa) vaccines. Neonatal DTPa vaccination induced strong pertussis-specific antibody and memory responses. Patterns of bacterial clearance were similar in both age groups. In contrast, as observed in human neonates, neonatal DTPw priming did not induce significant antibody responses to pertussis toxin (PT) and filamentous hemagglutinin (FHA) and even interfered with subsequent antibody responses. However, this did not reflect induction of permanent neonatal tolerance, as antigen-specific antibodies could be elicited by subsequent exposure to DTPa. Furthermore, despite these blunted PT and FHA […]

Reference


DOI : 10.1128/IAI.70.7.3521-3528.2002
PMID : 12065491

Available at:
http://archive-ouverte.unige.ch/unige:55966

Disclaimer: layout of this document may differ from the published version.
Immunogenicity and Protective Efficacy of Neonatal Vaccination against *Bordetella pertussis* in a Murine Model: Evidence for Early Control of Pertussis

Caroline Roduit,1 Paola Bozzotti,1 Nathalie Mielcarek,2 Paul-Henri Lambert,1 Giuseppe del Giudice,3 Camille Locht,2 and Claire-Anne Siegrist1*

World Health Organization Collaborating Center for Vaccinology and Neonatal Immunology, Department of Pathology and Pediatrics, University of Geneva, Geneva, Switzerland;1 Unité INSERM U447, Institut Pasteur de Lille, Lille, France;2 and Immunobiological Research Institute of Siena, Chiron S.p.A., 53100 Siena, Italy3

Received 26 December 2001/Returned for modification 18 February 2002/Accepted 15 April 2002

A significant resurgence of early cases of pertussis is being observed in infants too young to have yet completed their three-dose vaccination schedule. In this study, murine models of immunization and *Bordetella pertussis* challenge were adapted to early life. This allowed comparative evaluation of immunogenicity and protective efficacy of immunization initiated in the neonatal period (7-day-old mice) or in infancy (3-week-old mice) with diphtheria–tetanus–whole-cell pertussis (DTPw) and diphtheria-tetanus-acellular pertussis (DTPa) vaccines. Neonatal DTPa vaccination induced strong pertussis-specific antibody and memory responses. Patterns of bacterial clearance were similar in both age groups. In contrast, as observed in human neonates, neonatal DTPw priming did not induce significant antibody responses to pertussis toxin (PT) and filamentous hemagglutinin (FHA) and even interfered with subsequent antibody responses. However, this did not reflect induction of permanent neonatal tolerance, as antigen-specific antibodies could be elicited by subsequent exposure to DTPa. Furthermore, despite these blunted PT and FHA antibody responses, the protective efficacy of DTPa in neonatal mice proved similar to that in infant mice, resulting in complete bacterial clearance at day 8 after *B. pertussis* challenge. Thus, neonatal priming with antipertussis vaccines should be considered to reduce the window of vulnerability to pertussis at the time of its greatest severity.

Respiratory infection of young children with *Bordetella pertussis* causes whooping cough, a severe disease that is prevalent worldwide. Despite the administration of three doses of diphtheria-tetanus-pertussis vaccine (DTP) to 80% of children worldwide before their first birthday, recent World Health Organization (WHO) estimates indicate that whooping cough still annually affects up to 40 million children and results in 360,000 deaths (18). Although most of these infant deaths occur in developing countries, a significant shift of morbidity incidence towards young infants is also being observed in industrialized countries. In Canada, 78.9% of pertussis cases reported in 1999 were in children less than 6 months of age (with a median age of 12.4 weeks), and complications such as pneumonia, seizures, encephalopathy, and even death (0.9%) were common in this age group (16). Similar observations were reported in other countries with high pertussis vaccine coverage, attesting to an increased relative risk for infants too young to have yet completed their three-dose vaccination schedule (5, 11, 17, 31, 36, 38, 39).

New vaccine strategies are being considered to further improve pertussis control during the first months of life. Administration of adolescent and adult boosters is currently under discussion, with the hope of reducing the adult reservoir of *B. pertussis* (7, 10). However, this universal adult booster strategy is challenged by considerable logistic issues and high costs. In addition, the evaluation of the impact of an adult booster strategy on early infant disease and the estimation of the adult vaccine coverage that would be required to obtain a reduction in infant pertussis cases would require large, complex, and expensive efficacy studies. An alternative logical strategy would be to initiate infant vaccination earlier, i.e., in the neonatal period. Such a strategy was in fact considered decades ago (30), but its evaluation was halted by reports indicating poor immunogenicity of neonatal immunization with whole-cell pertussis vaccines, which even interfered with antibody responses to subsequent vaccine doses (2, 23, 30).

However, several elements prompted us to readdress the issue of neonatal pertussis immunization. First, the development of acellular pertussis vaccines demonstrating excellent safety and protective efficacy in young infants now offers an alternative to whole-cell pertussis immunization (13, 15, 20, 29, 32, 34, 35). Second, information can now be gathered from relevant preclinical models. Indeed, murine immunization and respiratory challenge models with *B. pertussis* have been established and have demonstrated excellent correlation with protective efficacy of diphtheria–tetanus–pertussis whole-cell (DTPw) and diphtheria-tetanus-pertussis acellular (DTPa) vaccines in human infants (14, 26, 28, 40). Third, extensive comparative analyses of the postnatal immune maturation taking place in humans and mice have recently demonstrated that the main developmental stages follow distinct kinetics but are strikingly conserved across species (reviewed in reference 33). Thus, the stage of immune maturation that prevails during the neonatal period (defined as 28 days in humans) may be ap-
proximated by that of a 1-week-old mouse, while immune responses of human infants (<1 year) are best reflected by those of 2- to 3-week-old mice.

In this study, we compared the pertussis-specific immunity and protective efficacy against B. pertussis respiratory challenge induced by neonatal (1 week) and infant (3 weeks) murine immunization with DTPa or DTPw.

MATERIALS AND METHODS

Bacterial antigens and vaccines. Genetically detoxified recombinant pertussis toxin (PT; 9K/129G), native pertactin (PRN), and filamentous hemagglutinin (FHA) prepared from B. pertussis and clinical-grade DTPa and DTPw vaccines were provided by Immunological Research Institute of Siena, Chiron SpA (Siena, Italy). A human dose of Chiron’s DTPa contains aluminum phosphate-adsorbed genetically detoxified PT (5 μg), PRN (2.5 μg), FHA (2.5 μg), and purified tetanus toxoid (TT; 60 μg) and diphtheria toxoid (30 μI). DTPw contains heat-killed B. pertussis and the same quantity of alum-adsorbed tetanus and diphtheria toxoids.

Mice and immunization. BALB/c mice were kept and bred under specific-pathogen-free conditions in the zootechnology unit of the Geneva University Medical Center. Breeding cages were checked daily for births, and pups were kept with mothers until weaning at the age of 4 weeks. Mice were immunized pathogen-free conditions in the zootechnology unit of the Geneva University Medical Center. Breeding cages were checked daily for births, and pups were kept with mothers until weaning at the age of 4 weeks. Mice were immunized subcutaneously in the upper back with 25% of the human dose of DTP or DTPw at the age of 3 weeks (infants) or 1 week (neonates) and boosted with the same formulation 3 weeks later, unless indicated otherwise. Research experiments were performed in compliance with federal guidelines and institutional policies.

Intranasal B. pertussis infection. Mice were challenged intranasally 2 weeks after the second immunization. Streptomycin-resistant B. pertussis BPSM (25) grown on a Bordet-Gengou agar (Difco, Detroit, Mich.) plate supplemented with 10% defibrinated sheep blood and 100 μg of streptomycin per ml was diluted in phosphate-buffered saline (PBS) to provide a challenge inoculum of 2.5 × 10^9 CFU/ml. Following intraperitoneal pentobarbital anesthesia, 20 μl of this bacterial suspension was administered slowly into each nostril. Groups of three to five mice were sacrificed 3 h after infection for quantification of the initial numbers of viable B. pertussis in the lungs and at 2, 5, and 8 days after challenge for determination of bacterial colonization. The lungs were removed and homogenized in PBS prior to dilution and plating of individual lung homogenates onto Bordet-Gengou agar plates. The number of CFU was counted after 4 days of incubation at 37°C. The ratio of the area under the clearance curve (AUC) of immunized and control mice during the course of infection, a measure of protective efficacy, was expressed as ΔAUC.

Determination of antigen-specific antibodies. Mice were bled 3 weeks after priming, 1 week after boosting, and then at regular intervals for determination of antigen-specific antibody responses. Antibody titers against PT, PRN, and FHA were measured by enzyme-linked immunosorbent assay (ELISA). Plates were coated with 1 μg of PT or 2 μg of PRN per ml overnight at 4°C or with 1 μg of FHA per ml for 3 h at 4°C. Alternatively, plates were coated with B. pertussis BPSM lysate (10 μg/ml) overnight at 4°C for determination of antibody titers against whole-cell bacterial antigens. After washing with PBS-0.05% Tween 20 and blocking with 1% bovine serum albumin, serial dilutions of sera were added to the plates and incubated at 37°C for 1 h.

For determination of antibody avidity, NH4SCN was added to the plates at 0.05% and interleukin-5 (IL-5) by capture ELISA, as previously described (3). In parallel, the numbers of antigen-specific IFN-γ or IL-4 secreting T cells in the spleen and lymph nodes were assessed by Elispot. Multiscreen 96-well nitrocellulose plates (Millipore, Molsheim, France) were coated overnight with rat anti-mouse IFN-γ or IL-4 monoclonal antibodies (5 μg/ml; Pharmingen, San Diego, Calif.). Spleen and lymph node cells were incubated at serial dilutions with heat-inactivated PT, PRN, FHA (0.2 to 5 μg/ml), or TT and incubated at 37°C under 5% CO2 for 72 h. The plates were then washed and incubated for 2 h at room temperature with 1 μg of biotinylated anti-mouse IFN-γ or IL-4 antibodies (PharMingen) per ml. After washing, the plates were incubated for 2 h at room temperature with conjugated Extravidin (Sigma, St. Louis, Mo.). Spots were developed by adding a substrate (3-amino-9-ethylcarbazole and 0.03% H2O2 in 0.1 M sodium acetate). The number of spots per well was counted with an Elispot reader (KFS Elispot; Zeiss, Munich, Germany), and the results were expressed as spots per 10^6 cells.

Statistical analysis. Statistical analyses of the results obtained in various groups of mice were performed with the Mann-Whitney U test. Differences with probability values of >0.05 were considered insignificant.

RESULTS

Comparative immunogenicity of DTPa and DTPw in infant mice. For our studies, we used an infant mouse immunization and sublethal intranasal challenge model recently shown to allow optimal detection of differences between human infant vaccines with clinically demonstrated higher or lower relative efficacy (6, 14). Infant (3-week-old) BALB/c mice were primed and boosted subcutaneously 3 weeks later with one-fourth of a human dose of the vaccines selected for this study: a tricomponent DTPa vaccine containing genetically detoxified PT, FHA, and PRN (13) and a DTPw vaccine from the same manufacturer.

As expected, DTPa and DTPw vaccination induced distinct patterns of responses to B. pertussis antigens (Fig. 1). DTPa elicited high IgG responses against PT and PRN and a moderate response to FHA. In contrast, high PRN and FHA antibody responses and a modest response to PT were induced by DTPw. T-cell responses were assessed 1 week after the second vaccine dose, following in vitro restimulation of splenocytes with individual B. pertussis vaccine antigens (heat-inactivated PT, PRN, or FHA) and TT as an internal control. TT-specific T cells were readily detected by Elispot following DTPa (382/10^6 IL-4 and 102/10^6 IFN-γ-producing cells) or DTPw (66/10^6 IL-4 and 27/10^6 IFN-γ-producing cells) immunization. In the same mice, T-cell responses to purified B. pertussis antigens were detected at lower frequencies (1 to 20/10^6 cells). Accordingly, B. pertussis antigen-specific IFN-γ and IL-5 release remained low in T-cell culture supernatants (data not shown). The number of B. pertussis antigen-specific T cells increased significantly (30 to 150/10^6 cells) following the administration of a third vaccine dose, confirming their presence and specificity (data not shown).

Two weeks after the second vaccine dose, mice were challenged intranasally with live B. pertussis. As expected, the number of bacteria in the lungs of naive control mice increased and remained high throughout the duration of the experiment (Fig. 2). In contrast, a rapid decline in bacterial numbers was observed in the lungs of mice previously immunized with either vaccine (Fig. 2). DTPw immunization conferred a high level of protection, resulting in complete bacterial clearance 8 days after challenge, whereas bacterial clearance proceeded at a slower pace following DTPa administration. Thus, immunization of infant BALB/c mice with the DTPa or DTPw vaccine
resulted in kinetics of bacterial clearance similar to those reported previously in adult mice for whole-cell and acellular pertussis vaccines (14, 27, 28, 40).

**Neonatal DTPa vaccination induces strong antibody responses.** To assess responses to neonatal vaccination, the priming dose of DTPa was given to 1-week-old mice, whose stage of immune maturity best approximates that of human newborns (33), and to 3-week-old infant mice as controls. The second vaccine dose was given after a 3-week interval. Neonatal DTPa immunization was well tolerated and did not affect weight gain, a sensitive marker of early-life vaccine-induced reactogenicity. Primary IgG1 responses to PT were only slightly lower than those elicited in infant mice and increased rapidly after boosting (day 28), indicating reactivation of neonatally primed PT-specific memory cells (Fig. 3). Levels of anti-PRN and anti-FHA antibodies were similar regardless of age at immunization. Although neonatal T-cell responses were too low to determine cytokine responses (data not shown), the ratio of IgG1 to IgG2a was similar in mice immunized at 1 and 3 weeks of age, with a strong predominance of IgG1 in both age groups (Fig. 3). Thus, DTPa vaccination elicited antibody responses as strong in 1-week-old as in infant mice.

**Antibody responses to DTPw vaccination in the neonatal period.** In a parallel series of experiments, BALB/c mice were primed with DTPw vaccine at 1 week (neonatal period) or at 3 weeks (infant controls) of age and boosted 3 weeks later. Neonatal DTPw vaccination was well tolerated, and weight gain remained normal after immunization. In contrast to responses observed following neonatal DTPa vaccination, neonatal anti-PT and anti-FHA IgG1 and IgG2a responses were barely detectable and remained significantly weaker than infant responses even after boosting (Fig. 4.). It should be noted that a single dose of DTPw elicited higher PT and FHA antibody titers in 3-week-old infant mice than a booster dose of DTPw given to 4-week-old mice previously primed at 1 week of age. This inhibition of subsequent PT and FHA antibody responses by neonatal priming contrasted to age-independent PRN responses. It also contrasted with the induction of similar antibody responses against *B. pertussis* antigens following either neonatal or infant DTPw immunization (day 35: IgG1, 4.47 ± 0.18 and 4.51 ± 0.22 log10, and IgG2a, 3.98 ± 0.21 and 3.42 ± 0.26 log10 following neonatal and infant immunization, respectively). Neonatally induced T-cell responses were too low to determine cytokine responses (data not shown), but the ratio of IgG1 to IgG2a was similar regardless of age at immunization (Fig. 4).

The observed inhibition of subsequent vaccine responses to PT following neonatal DTPw administration, which had already been reported in human newborns (2, 23, 30), prompted us to investigate whether neonatal DTPw immunization induces permanent immune tolerance to this vaccine antigen. Following neonatal priming and boosting at 4 weeks of age with DTPw, mice were allowed to rest until 12 weeks of age and were then given a single dose of DTPa, which contains a higher quantity of PT in a more immunogenic form. Mice were bled before and 7 days and 3 weeks after the DTPa dose to compare their anamnestic PT-specific IgG response to that of naive adult controls immunized with a single dose of DTPa.

At the time of DTPa administration, only 3 of 24 neonatally primed mice had PT-specific IgG1 titers of >2.5 log10 and the average antibody titers were very low (median, 2.0 log10) (Fig.
5A). Seven days after DTPa administration, PT-specific IgG levels remained low in most mice (median, 2.54 log₁₀) and not statistically different from those of control mice, i.e., without evidence of any anamnestic response to PT. In contrast, a significant increase in anti-PT antibodies became apparent 3 weeks after DTPa, with titers similar (4.7 log₁₀) to those of adult controls (Fig. 4). At this 21-day time point, the avidity index of PT-specific antibodies, determined by an ELISA elution assay with thiocyanate as a chaotropic agent and used as an additional surrogate marker for induction of PT-specific memory B cells, was similar (1.50 M) in both immunization groups. In contrast, a positive anamnestic response to PRN was observed in neonatally primed mice, indicated by a significant increase in IgG titers at day 7 after DTPa administration compared to primary responses elicited in control mice (Fig. 5B).

Altogether, these observations demonstrate that in 1-week-old mice, DTPw priming had failed to generate PT-specific B-cell responses and had interfered with responses to subsequent doses of DTPw but did not result in permanent tolerance to PT.

**Protective efficacy of neonatal DTPa and DTPw vaccination.**

The significantly different immunogenicity of neonatal DTPa and DTPw vaccines prompted us to compare their respective protective efficacy. BALB/c mice were immunized with DTPa or DTPw vaccine at 1 and 3 weeks of age and boosted 3 weeks later with the same vaccine formulations. Challenge was performed 2 weeks later by intranasal inoculation of live *B. pertussis*. As expected, bacterial counts increased in the lungs of nonimmunized challenged mice (Fig. 6). In contrast, bacterial clearance occurred rapidly following neonatal DTPa immunization (Fig. 6A). In accordance with our observation of similar vaccine responses, the same level of protective efficacy was observed following neonatal and infant DTPa immunization.

Remarkably, neonatal DTPw immunization also showed excellent protective efficacy (Fig. 6B). Despite undetectable or low anti-PT and anti-FHA antibodies at the time of challenge (Fig. 3 and data not shown), the range of early (day 2) bacterial decline was similar in mice immunized at 1 and 3 weeks of age. Moreover, despite slightly higher bacterial counts at day 5, bacterial clearance was similar in both age groups and achieved earlier than following neonatal or infant DTPa vaccination. This was confirmed by calculation of similar ∆AUC values following neonatal (∆AUC, 31.2 ± 5.0) and infant (∆AUC, 36.0 ± 4.4) DTPw immunization or neonatal (∆AUC, 25.5 ± 3.1) and infant (∆AUC, 27.2 ± 1.7) DTPa immunization, respectively. Altogether, these data demonstrate the strong protective efficacy of neonatally induced murine vaccination with...
either the DTPa or DTPw vaccine, with a somewhat better protective effect of DTPw vaccination in both age groups.

DISCUSSION

This study provides evidence supporting the capacity of both DTPw and DTPa neonatal vaccination to allow earlier induction of protection against pertussis despite age-dependent limitations of vaccine-induced responses. It also demonstrates that murine models of DTPw vaccination and *B. pertussis* respiratory challenge may be adapted to the neonatal period so as to reproduce immunological features previously observed following neonatal DTPw vaccination in humans.

The protective efficacy of neonatal DTPw and DTPa vaccination was assessed in a murine immunization-intranasal challenge model known to discriminate between vaccines of distinct protective efficacies in human infants (6, 14). This discriminating capacity was confirmed by the observation of a more rapid lung bacterial clearance (day 8) in infant mice immunized with a whole-cell rather than a three-component (PT, FHA, and PRN) acellular pertussis vaccine containing genetically inactivated PT. This result is in accordance with previous observations obtained in adult mice (reviewed in reference 26) and is considered to reflect DTPa and DTPw induction of distinct patterns of cellular and humoral immune responses to *B. pertussis* antigens. Thus, the immunization and intranasal pertussis challenge model used in this study is capable of detecting differences between vaccines of higher and lower relative efficacies, a prerequisite for our evaluation of alternative vaccination strategies.

Comparative analyses of human and murine responses to a panel of infant vaccines have indicated the existence of strikingly conserved patterns of immune maturation, including age-dependent characteristics of antibody and T-cell responses, suggesting significant conservation of pre- and postnatal developmental stages across species (reviewed in reference 33). However, characteristic features of neonatal immune responses have indicated that immune maturation is significantly more advanced in human neonates than in newborn mice, which is likely to reflect in part the more advanced development of human secondary lymphoid organs at the time of birth. It was thus suggested that the stage of immune maturation of human newborns may best be approximated by that of a 7-day-old mouse (3). This correlation is strongly supported by the observation that DTPw immunization initiated in a 7-day-old mouse results in a serological pattern similar to that seen

![FIG. 4. Antibody responses to neonatal DTPw immunization. Mice were primed at 1 week of age (or at 3 weeks for the controls) and boosted 3 weeks later with DTPw, PT-, FHA-, and PRN-specific IgG1 (A) and IgG2a (B) responses were measured by ELISA in individual sera at days 20 (primary response), 28, and 42 (secondary responses) after priming. Titers are expressed as means and standard deviations in ELISA units of six to eight mice per group. *, P < 0.05](image-url)
following human neonatal (<7 days) DTPw vaccination (23, 30).

We show here that neonatal murine DTPw priming does not result in detectable anti-PT and anti-FHA responses and does interfere with responses to subsequent DTPw vaccine doses, so that the secondary responses at 4 weeks of age remained weaker than the primary responses of 3-week-old mice. This strikingly similar to the observation that human neonatal DTPw vaccination did not elicit antibody responses and did interfere with antibody responses to subsequent doses of DTPw, in contrast to efficient primary responses of 4-week-old infants (23, 30). This phenomenon, which was considered to reflect "immunological paralysis," was further confirmed by the observation of a significant limitation of anti-PT IgG responses to DTPw immunization (given at 2, 4, and 6 months of age) in infants who had been primed with DTPw in the neonatal period (2). This inhibition was also present in a subset of infants with low titers of cord blood B. pertussis-specific maternal antibodies, excluding interference by antibodies of maternal origin, and remained unexplained.

Observations made in our murine model of neonatal DTPw immunization indicate the induction of a transitory unresponsiveness rather than persisting tolerance. When mice primed with DTPw at 1 week (and boosted 3 weeks later) were exposed at 12 weeks of age to recombinant PT, PT-specific antibodies remained low at day 7 postimmunization. This lack of anamnestic response confirmed that neonatal administration of DTPw had interfered with priming of PT-specific cells. However, DTPa-induced PT-specific IgG subsequently reached levels similar to those elicited in naive adult controls (Fig. 5), demonstrating that PT-specific cells had not been made permanently tolerant in the neonatal period. This transient unresponsiveness to subsequent antigenic stimulation is antigen specific, i.e., affected PT and FHA but not PRN or total antigens from a B. pertussis cellular lysate, and its detailed immunological bases (i.e., relative T- and B-cell unresponsiveness) are currently being studied.

It is remarkable that despite limited antibody responses to PT and FHA, the protective efficacy of neonatally initiated DTPw immunization was nevertheless similar to that of control infant mice. The early (day 2) bacterial clearance is currently considered as essentially reflecting antibody-mediated protection (26). That it occurs in the absence of anti-PT and anti-FHA antibodies suggests an important role for other antibodies. This could reflect the importance of PRN-specific antibodies, as previous studies showed much faster lung bacterial clearance following murine immunization with a tricomponent (PT, FHA, and PRN) compared to the bicomponent (PT and FHA) vaccine from the same manufacturer (6) or following administration of PRN immune serum to adult mice (28). In human infants, superior protective efficacy was also

FIG. 5. Antibody responses to DTPa vaccine after neonatal DTPw immunization. Mice (n = 24) immunized at 1 and 4 weeks of age with DTPw and naive adult controls (n = 10) were immunized at 12 weeks of age with a single dose of DTPa vaccine. PT-specific IgG responses were measured by ELISA in individual sera 1 day before challenge and on days 7 and 21 after challenge. Titers are expressed as medians and standard deviations in ELISA units.

FIG. 6. Kinetics of B. pertussis clearance from the lungs after respiratory challenge of mice following neonatal immunization with DTPw or DTPa. Groups of BALB/c mice were immunized subcutaneously at 1 week (neonatal) or 3 weeks (infant controls) of age with 25% of the human dose of DTPa (A) or DTPw (B) or with PBS (controls). Two weeks after the second immunization, mice were challenged intranasally with B. pertussis and sacrificed at various intervals for evaluation of CFU counts on individual lung homogenates. Results are expressed as means (± standard deviations) of viable B. pertussis counts from three to four mice per group at each time point.
observed for the three- than for the two-component vaccine (13, 15). Moreover, two clinical studies also showed some correlation between the level of PRN-specific antibodies and clinical protection (8, 37), although a single antigen-protective antibody threshold could not be defined. Alternatively, however, early (day 2) bacterial decline following neonatal DTpW immunization could reflect clearance by antibodies elicited to other B. pertussis antigens which were present at the time of challenge at titers similar to those seen following infant DTpW immunization.

It is also remarkable that neonatally initiated DTpW immunization resulted in complete bacterial clearance just 8 days after challenge. Such rapid clearance by day 8 was also observed following adult DTpW and not DTpA immunization (26). As administration of IL-12 at the time of DTpA immunization was shown to increase DTpA-induced bacterial clearance to the level of DTpW (22), a role for DTpW-induced Th1 cells in the clearance of intracellular organisms was suggested. Under our experimental conditions, neonatal T-cell responses to purified PT, FHA, and PRN antigens remained too weak for reliable evaluation of cytokine patterns, but similar mechanisms might be involved despite the preferential induction of neonatal murine responses towards the Th2 differentiation pathway (3). This could reflect the potent IL-12-inducing capacity of B. pertussis endotoxin (22), which is present at low levels in the DTpW vaccine.

In contrast to the limited and age-dependent responses to DTpW, vaccination with DTpA initiated either in the neonatal period or in infancy resulted in similarly strong induction of pertussis-specific antibody-secreting and memory cells. Accordingly, early (day 2) antibody-mediated clearance of B. pertussis was similar regardless of age at immunization, while similar kinetics during the second part (days 5 to 8) of the bacterial clearance provide indirect evidence for a similar induction of T-cell responses. This is in line with the fact that DTpA vaccines are known to preferentially induce Th2 responses (reviewed in reference 26), which are easily elicited in early life (33).

In conclusion, this study sets the stage for further clinical evaluation of neonatal priming against B. pertussis to reduce the window of vulnerability to pertussis in young infants. It demonstrates that immunization with either vaccine beginning in the neonatal period was as efficacious with regard to protection against challenge as immunization beginning in infancy and thus resulted in earlier induction of protection. It must be noted, however, that the rapid development of the mouse did not allow us to determine whether protection can be established during the “neonatal period.” Neonatal immunization is already routinely implemented (Mycobacterium bovis BCG, oral polio, and hepatitis B vaccines) in many developing countries where the death toll from pertussis is particularly high.

Our observations suggest that the inhibition of anti-B. pertussis antibody responses previously observed following neonatal DTpW priming does not imply induction of long-lasting immune tolerance or the absence of protective efficacy. Given its excellent safety and efficacy profiles, DTpA could be preferred for administration of a neonatal priming dose prior to the currently recommended three-dose series. Although immune immaturity is expected to limit early-life antibody responses, it is known to allow early induction of memory B and T cells in human newborns (1, 4, 9, 19). Thus, neonatal priming should reduce the window of vulnerability to pertussis at the time of its greatest severity.

ACKNOWLEDGMENTS

This work was supported by grants and fellowships from the DG XII Fifth Programme Framework (grant NEOVAC ref QLK2-CT-1999-00429) and OFES (ref 99.0082), from the Swiss National Research Foundation, and from the Fondation Marcel Merieux.

We thank Paolo Quiriqghetti for assistance with animal care, Christine Brighetto for excellent secretarial assistance, and Samuele Pellepoli (Chiron SpA, Siena, Italy) for constantly ensuring the availability of reagents and vaccines for this study.

REFERENCES