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Helminth- and Bacillus Calmette-Guérin-Induced Immunity in Children Sensitized In Utero to Filariasis and Schistosomiasis

Indu Malhotra,* Peter Mungai,† Alex Wamachi,‡ John Kioko,† John H. Ouma,† and James W. Kazura,* and Christopher L. King2†§

Infants and children are routinely vaccinated with bacillus Calmette-Guérin (BCG) in areas of the world where worm infections are common. Because maternal helminth infection during pregnancy can sensitize the developing fetus, we studied whether this prenatal immunity persists in childhood and modifies the immune response to BCG. Children and newborns living in rural Kenya, where BCG is administered at birth and filariasis and schistosomiasis are endemic, were examined. T cells from 2- to 10-year-old children of mothers without filariasis or schistosomiasis produced 10-fold more IFN-γ in response to mycobacterial purified protein derivative than children of helminth-infected mothers (p < 0.01). This relationship was restricted to purified protein derivative because maternal infection status did not correlate with filarial Ag-driven IL-2, IFN-γ, IL-4, or IL-5 responses by children. Prospective studies initiated at birth showed that helminth-specific T cell immunity acquired in utero is maintained until at least 10–14 mo of age in the absence of infection with either Wuchereria bancrofti or Schistosoma haematobium. Purified protein derivative-driven T cell IFN-γ production evaluated 10–14 mo after BCG vaccination was 26-fold higher for infants who were not sensitized to filariae or schistosomes in utero relative to subjects who experienced prenatal sensitization (p < 0.01). These data indicate that helminth-specific immune responses acquired during gestation persist into childhood and that this prenatal sensitization biases T cell immunity induced by BCG vaccination away from type 1 IFN-γ responses associated with protection against mycobacterial infection. The Journal of Immunology, 1999, 162: 6843–6848.

Bacillus Calmette-Guérin (BCG) is one of the most widely used vaccines in the world (1). Although a single vaccination induces delayed-type skin reactivity and in vitro T cell responses to mycobacterial purified protein derivative (PPD), its efficacy in protecting against tuberculosis is controversial and variable (2). There may be several explanations for the apparent discrepancy of results from different clinical trials, such as the strain of BCG used, geographic variability in the virulence of Mycobacterium tuberculosis, HLA restriction of immunity, and interference with protection that arises from coinfection with other mycobacterial species. Metaanalysis of published trials indicates that the efficacy of BCG vaccination against clinical tuberculosis is less in tropical than temperate regions of the world (3). Because filariasis, schistosomiasis, and other worm infections are prevalent in areas of the tropics where newborns and infants are vaccinated with BCG, it is possible that immunologic responses induced by these or other helminthic infections biases immunity and diminishes the efficacy of vaccination.

Studies of experimental animals have shown that the immune environment established by prior sensitization to helminths influences immunity to unrelated Ags. Following immunization with sperm whale myoglobin, mice with preexisting Schistosoma mansoni infection develop Ag-specific T cells that produce IL-4, whereas T cells from uninfected animals generate relatively more IFN-γ and IL-2 (4). With respect to the impact of helminthiases on the host response to intracellular pathogens, resistance against viral infections that is dependent on cytokines such as IFN-γ is compromised in mice with schistosomiasis (5). Although the interaction between heminthic and mycobacterial infections in experimental animals has not been studied, mice immunized with PPD following the induction of strong type 2 responses by the filarial parasite Brugia malayi develop T cell cytokine responses that are skewed away from the type 1 and toward the type 2 pattern. Simultaneous immunization of naive animals with helmint and mycobacterial Ags does not diminish IFN-γ or enhance type 2 cytokine responses to PPD (6).

Observations by others and us indicate that newborns whose mothers have schistosomiasis or bancroftian filariasis during pregnancy are frequently sensitized to these helminths in utero (7–11). Consistent with the propensity of worms to induce allergic responses, cord blood lymphocytes (CBL) from newborns of helminth-infected mothers produce IL-4, IL-5, and IgE as well as IFN-γ (10, 11). The present study tests the hypothesis that in utero sensitization to helminths establishes immunologic memory that persists into childhood and biases the T cell cytokine response induced by BCG vaccination. Two approaches were taken to examine this interaction in residents of rural Kenya, where BCG is administered at birth and filariasis and schistosomiasis are endemic. First, in a cross-sectional study, PPD- and filarial Ag-driven cytokine responses by PBMC from 2- to 10-year-old children living in villages where bancroftian filariasis is endemic were evaluated and compared according to the filarial infection status of

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3 Abbreviations used in this paper: BCG, bacillus Calmette-Guérin; PPD, purified protein derivative of Mycobacterium tuberculosis; CBL, cord blood lymphocytes; BmA, soluble Ag preparation of Brugia malayi parasites; SWAP, soluble Ag preparation of adult Schistosoma haematobium parasites.
their mothers. Second, in a prospective study initiated at birth, the patterns of helminth- and PPD-driven cytokine responses by 10- to 14-mo-old infants without or with documented in utero sensitization to Wuchereria bancrofti and Schistosoma haematobium were compared.

Materials and Methods

Cross-sectional study of 2- to 10-year-old children and their mothers

Thirty-three mother-child pairs living in two villages, Darigube and Eshu, participated. W. bancrofti infection is endemic and S. haematobium prevalence is low in these villages (6.3% of 15- to 40-year-old women had S. haematobium eggs in a single urine sample). S. mansoni is not transmitted in the study area. According to the presence of nocturnal microfilaria and filarial antigenemia (see below), 16 children had mothers who were not infected and 17 had mothers with filariasis. None of the women were passing S. haematobium eggs in their urine or S. mansoni eggs in their feces.

Prospective study of infants from birth to 10–14 mo of age

Twenty-five newborns delivered in the facilities of Msambweni Hospital in 1996 and 1997 were enrolled. CBL responses to filarial Ag (B. malayi adult worm extract, BmA) and S. haematobium adult worm Ag (SWAP), and the helminth infection status of mothers were evaluated as described below. Patients who attend Msambweni Hospital come from a wide area of Coast Province that includes villages where both bancroftian filariasis and schistosomiasis haematobia are endemic.

BCG vaccination

The study subjects were vaccinated with BCG (Staten Serum Institute, Copenhagen, Denmark) at birth in accordance with the policy of the Kenya Ministry of Health. BCG vaccination of 2- to 10-year-old children was inferred from health records and by the presence of a typical scar on the upper arm. In the case of newborns, administration of BCG within 24 h of birth was observed by the investigators and confirmed by the presence of a scar at 10–14 mo of age.

Informed consent and ethical approval

Verbal informed consent for participation of children and newborns was obtained from their mothers. Ethical approval was secured from the Kenya Ministry of Health and Human Investigation Institutional Review Board at University Hospitals of Cleveland, Case Western Reserve University.

Parasitologic determinations

To diagnose S. haematobium infection, 10 ml of urine was passed through a polycarbonate membrane (Nuclepore, Pleasanton, CA) and examined microscopically for ova. Stool samples were examined for intestinal helminth infection status of mothers were evaluated as described below. Patients who attend Msambweni Hospital come from a wide area of Coast Province that includes villages where both bancroftian filariasis and schistosomiasis haematobia are endemic.

Ags and mitogens

BmA and SWAP were prepared as described (14, 15). The preparations contained <0.5 ng/ml endotoxin (5- to 50-fold less than that required for LPS stimulation of cytokine production by human lymphocytes) (16). PPD was purchased from Evans Medical Institute (Leatherhead, Surrey, U.K.).

Isolation of PBMC and culture conditions for in vitro cytokine production

Depending on the age and size of the child, 2–15 ml of blood was obtained by venipuncture. PBMC were separated from heparinized cord or venous blood by density gradient centrifugation on Ficoll-Hypaque and resuspended in RPMI 1640 supplemented with 10% FCS, 4 mM L-glutamine, 25 mM HEPEs, and 80 μg/ml gentamicin (BioWhittaker, Walkersville, MD) (C-RPMI). PBMC were incubated at 2 × 10^6 cells/ml in C-RPMI in a total volume of 1 ml. Media alone, SWAP (50 μg/ml), BmA (10 μg/ml), PPD (1:200 dilution), or PMA (50 ng/ml) plus ionomycin (1 μg/ml; Calbiochem, La Jolla, CA) were added to duplicate cultures. Cells were incubated at 37°C in 5% CO₂. Supernatants were collected at 36 h (for IL-2 and IL-4 detection) and 72 h (for IL-5 and IFN-γ measurement) and immediately frozen at −70°C for subsequent measurement of cytokine production.

Cytokine and Ig ELISAs

Cytokine levels in culture supernatants were measured by ELISA and expressed in pg/ml by interpolation from standard curves based on recombinant lymphokines using Abs and methods as described (17). Monoclonal Abs for capture and biotinylated Abs, respectively, for detection of each cytokine were: IL-5, TRFK5 and 5D10 (PharMingen, San Diego, CA); IFN-γ, 7D4 (PharMingen); IL-4, 8D and 2D2 (PharMingen); IFN-γ, M-700 and M-701 (Endogen, Cambridge, MA); and IL-10, 18551D and 18652D (PharMingen). The limits of detection were 18 pg/ml for IL-5, 16 pg/ml for IL-4, 10 pg/ml for IFN-γ, and 16 pg/ml for IL-10. Polyclonal IgE and BmA- and SWAP-specific IgE and IgG4 Abs were quantified as described (14, 18, 19).

Statistics

Results are expressed as the mean ± SEM using log-transformed data unless otherwise stated. Log-transformation of cytokine levels produced a normal distribution of the data. Thus, a comparison of means of between the two independent populations was examined by the Student’s t test. We also compared differences between populations using the nonparametric Mann-Whitney U test. All comparisons were also statistically significant using this test except for PPD-driven IL-5 production by infants born to infected vs noninfected mothers in Fig. 1b. The frequencies of responses...
Results

Cross-sectional study: relationship between maternal filarial infection and filarial Ag and PPD-driven cytokine responses by 2- to 10-year-old children

Because observations made in some endemic areas suggest that maternal filarial infection leads to tolerization to filarial Ags in offspring (20–22), the relationship between maternal W. bancrofti infection and childhood infection status and filarial (BmA)-driven cytokine responses was first examined. Maternal infection status did not correlate with BmA-driven IFN-γ or IL-5 production by children. PBMC from 12 of 16 children of uninfected mothers made IFN-γ compared with 9 of 17 children of W. bancrofti-infected mothers (p > 0.10; Fig. 1a). The geometric mean levels of BmA-driven IFN-γ and IL-5 production by children in each group were also similar. Of note, filarial Ag-driven IL-5 production was significantly greater than IFN-γ production (e.g., children of filarial-infected mothers produced 160 pg/ml of IL-5 and 32 pg/ml of IFN-γ; p < 0.01).

In contrast to BmA-driven responses, PPD-specific cytokine production differed according to the filarial infection status of the child’s mother (Fig. 1b). PBMC from 15 of 16 children (94%) of uninfected mothers made IFN-γ when stimulated with PPD compared with 8 of 14 children (57%) of infected mothers (p = 0.03). Moreover, the mean level of PPD-driven IFN-γ production by PBMC from children in the former group was 10-fold higher than the latter (p < 0.01). With respect to PPD-driven IL-5, the frequency of responses was similar for PBMC from children in both groups (50% and 57%, respectively). However, among children whose PBMC did produce IL-5 when stimulated with PPD, those from mothers with W. bancrofti infection generated significantly higher amounts of this cytokine (means of 211 pg/ml vs 82 pg/ml, p = 0.03). PPD-driven IFN-γ and IL-5 or IL-10 production were not inversely correlated (data not shown), suggesting that cross-regulation of type 1 and type 2 cytokine production was not occurring in vitro (23).

Young children in these filarial endemic communities rapidly became infected with filariasis as determined by the presence of filarial antigenemia. None of the 2-year-old children studied were infected. Overall, 10 of the 37 children (27%) between 2–10 years old (median, 6 years) were infected. There was no association between the filarial infection status of mothers and children (25% of children of uninfected mothers had filarial antigenemia compared with 36% of children of infected mothers; p > 0.05).

Prospective study: acquisition of filarial or schistosome infection between birth and 10–14 mo of age

To determine whether infants were infected with W. bancrofti or S. haematobium between birth and 10–14 mo of age, blood was examined for the presence of filarial Ag and urine was evaluated for schistosome eggs at both time points. None of the subjects were infected with either parasite by these criteria. One infant had hookworm ova in her stool at 14 mo of age.

Two additional examinations were performed to determine whether the infants acquired W. bancrofti or S. haematobium infection during the course of the study. First, the levels of parasite-specific IgE at birth (i.e., in cord blood) and at 10–14 mo of age were compared. BmA-specific IgE decreased in 21 of 25 cases and increased in only 4 paired samples, although these increases were <4-fold (Fig. 2). Similar results were obtained for SWAP-specific IgE, although none increased with age (data not shown). The lack of increase in BmA- and SWAP-specific IgE was not due to an inability to generate this Ig isotype because the level of polyclonal IgE increased 2- to >100-fold during this time interval in all of the subjects (Fig. 2). Second, BmA- and SWAP-specific IgG4 Abs at 10–14 mo of age were measured. None of the subjects had parasite-specific Abs of this IgG subclass (IgG4 Abs in cord blood were not evaluated because these are primarily of maternal origin). In aggregate, these data indicate that none of the subjects were infected with W. bancrofti or S. haematobium between birth and 10–14 mo of age.

In utero sensitization to S. haematobium and W. bancrofti and persistence of helminth-specific T cell immunity during infancy

Similar to earlier observations of a different group of infants delivered at the same hospital (10), 12 of the 25 newborns in the current study were sensitized to S. haematobium and/or W. bancrofti in utero (i.e., their CBL produced IL-2, IFN-γ, IL-4, and/or IL-5 when stimulated with BmA or SWAP; Table I). Newborns whose mothers had filariasis or schistosomiasis were more likely to have helminth Ag-driven CBL cytokine responses than those whose mothers were not infected with either parasite (75% vs 23%, respectively; p < 0.03).

Data describing BmA- and SWAP-driven cytokine responses by PBMC from 10- to 14-mo-old infants according to the corresponding CBL responses are also presented in Table I. Peak cytokine response is shown to either Ag. Four infants responded to both Ags at birth. The responses to schistosome and filarial Ags were combined because either helminth infection stimulates an allergic-type immune response capable of biasing the immune response to BCG vaccination. Four of the 13 infants (38%) whose CBL responses indicated they were not sensitized to either helminth at birth had BmA- or SWAP-driven responses at 10–14 mo. PBMC from these four individuals made IFN-γ and two also made IL-5. Two subjects (numbers 170 and 226) had mothers with filarial or schistosome infection. Subject 226 also had elevated SWAP-specific IgE in his cord blood (13.7 ng/ml vs <0.8 ng/ml for cord blood from 20 North American controls), suggesting that he was sensitized to S. haematobium at birth despite the lack of a detectable helminth-driven CBL response. In contrast to subjects who lacked evidence of prenatal sensitization, 10 of 12 (83%) newborns whose CBL produced one or more cytokines when stimulated with BmA or SWAP retained Ag-specific lymphocyte responses at 10–14 mo of age (p < 0.05; Table I). Notably, nine of these infants had mothers with elevated parasite-specific IgG4 Abs, i.e., suggesting that some mothers were infected during the time of the child’s gestation. The two subjects in whom T cell responses were not detectable at 10–14 mo of age (numbers 64 and 65) had helminth-driven IL-2

FIGURE 2. BmA-specific and polyclonal IgE levels in plasma obtained at birth and 10–14 mo of age. Each pair of points (open circles, cord blood; closed circles, blood from 10– to 14-mo-old infants) connected by a line represents BmA-specific or polyclonal IgE levels for a single subject. Note the differences in scales for BmA-specific and polyclonal IgE.
responses at birth. With respect to the 10 infants who retained helminth Ag-specific responses, type 2 cytokines increased with age in all cases. The levels of IFN-γ and IL-2 production did not show a consistent trend with increasing age.

In utero sensitization to helminths and PPD-driven cytokine responses at 10–14 mo of age

PBMC from all 13 infants whose CBL responses indicated they were not sensitized to *W. bancrofti* or *S. haematobium* at birth produced PPD-driven IFN-γ at 10–14 mo of age (Fig. 3a). Cells from four of these infants also produced IL-5 in response to PPD. Two of the latter subjects (numbers 226 and 170) had evidence of in utero sensitization to helminths (see Table I). Cytokine responses by infants whose CBL demonstrated they were sensitized to the helminths in utero showed a different pattern of responses. The mean level of IFN-γ produced by this group was only 3.8% of that of infants who were not sensitized in utero (respective means of 88 pg/ml and 2270 pg/ml; *p* < 0.01). PBMC from three subjects in the helminth-sensitized group did not produce IFN-γ when stimulated with PPD. With respect to IL-5, PBMC from 9 of 12 infants sensitized to helminths at birth generated IL-5 (*p* < 0.01 compared with the unsensitized group).

The relationship between the pattern of helminth Ag-driven cytokine production at birth and PPD-driven cytokine production at 10–14 mo of age is described in Fig. 3b. Infants whose CBL showed a dominant type 2 response to helminth *Ag* (ratio of type 1:type 2 response, <1:0) had PBMC that produced relatively little or no IFN-γ when stimulated with PPD. In contrast, PBMC from infants whose CBL produced exclusively or relatively more IFN-γ or IL-2 in response to SWAP or BmA generated greater amounts of PPD-driven IFN-γ.

PPD-driven cytokine responses by CBL were also evaluated. Twenty-two of the 25 CBL preparations did not produce IFN-γ, IL-2 or IL-5.

![FIGURE 3.](http://www.jimmunol.org/)

**FIGURE 3.** PPD-driven IFN-γ production at 10–14 mo of age for infants according to whether or not they were sensitized to *W. bancrofti* or *S. haematobium* in utero. *a*, Subjects were categorized according to whether or not their CBL produced one or more cytokines when stimulated with BmA or SWAP (closed circles, not sensitized; open circles, sensitized). The newborns were vaccinated with BCG on the day of their delivery, and the production of cytokines by PBMC stimulated with PPD was evaluated 10–14 mo later. The mean level of PPD-driven IFN-γ produced at 10–14 mo of age was greater for infants who were not sensitized than that of infants who were sensitized in utero (*p* < 0.01). Both cytokine levels (*p* = 0.02; Student’s *t* test of log-transformed data) and frequency (4 of 13 vs 9 of 12; *p* = 0.03) of PPD-specific IL-5 was less in the not sensitized than the sensitized group. The horizontal bars indicate geometric means. *b*, Relationship between the ratio of filarial- and/or schistosome Ag-driven type 1 (IFN-γ or IL-2) to type 2 (IL-4 or IL-5) cytokine production by CBL and PPD-driven IFN-γ by PBMC obtained 10–14 mo after BCG vaccination. Closed circles indicate that these CBL made IL-5 or IL-4 as well as IFN-γ. Closed squares indicate that these CBL made IFN-γ but neither IL-4 nor IL-5.

Table I. Cytokine responses to helminth antigens at birth and 10–14 mo of age in infants who were not sensitized or who were sensitized in utero to *W. bancrofti* and/or *S. haematobium*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Net IFN-γ Production (pg/ml)*</th>
<th>Net IL-5 Production (pg/ml)**</th>
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<tbody>
<tr>
<td></td>
<td>Cord blood</td>
<td>PBMC collected at 10–14 mo</td>
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<tr>
<td>Not Sensitized</td>
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<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
</tr>
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</tr>
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</tr>
<tr>
<td>305*</td>
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* Lymphocytes prepared from all subjects were stimulated with both filarial *Ag* BmA and SWAP. Cytokine responses to filarial *Ag*s are shown in subjects 28, 179, 188, 218, 305, and 342 (indicated by †). All other *Ag*-induced cytokine responses were in response to SWAP. Some individuals showed cytokine responses to both filarial and schistosome *Ag*s (numbers 28, 47, and 305). In these cases, the *Ag* that stimulated the greatest levels of cytokine production is shown. Values represent the means of duplicate cultures (variation was <22% between replicate cultures) of 2 × 10^4 *lymphocytes/ml* collected at 36 h for IL-2 and IL-4 and 72 h for IFN-γ and IL-5.

† For sensitized subjects, the net IFN-γ or IL-2 production (pg/ml) was measured.

‡ For sensitized subjects, the net IL-5 or IL-4 production (pg/ml) was measured.

† For sensitized subjects, net IFN-γ or IL-2 production (pg/ml) was measured.

*304a and 304b are dizygotic twins.*
IL-2, IL-4, or IL-5 when stimulated with PPD; three samples produced one or more of the cytokines. These responses did not correlate with PPD-driven cytokine production reevaluated 10–14 mo later.

Discussion

The current study demonstrates that prenatal sensitization to filariae and schistosomes occurs in ~50% of newborns examined in this area of Kenya, that immunologic memory to the helminths persists during infancy, and that this prenatal sensitization is associated with diminution of type 1 immunity induced by BCG vaccination. The observations of filarial and schistosomiasis-specific immunity in infants and children are notable in several respects. First, helminth Ag-specific T cell responses demonstrable at birth (i.e., by measurement of CBL cytokine production) persist for at least 10–14 mo, even in the absence of boosting afforded by infection. This finding supports the notion that immunologic memory established by priming of prenatal T cells with Ags that pregnant women encounter through infection or vaccination (24–28) is long-lived, and that T or B cell responses detected in cord blood preparations are not due to maternal lymphocytes that have passed into the fetal circulation. Second, the lack of evidence for acquisition of *W. bancrofti* or *S. haematobium* infection in infants <14 mo old suggests that prenatal sensitization rather than exposure to these helminths during childhood is important in determining the initial immune response elicited by natural infection. It is thus possible that infants who have been sensitized to filariae before birth may develop anamnestic responses to the same parasite Ags following infection. We speculate that this situation is analogous to reports demonstrating that vaccination of pregnant women with tetanus toxoid leads to enhanced Ab responses of infants following primary immunization (26). Because the methods used to ascertain infection status (parasitologic diagnosis, IgG4 Abs, antigenemia) may not be sensitive enough to detect extraordinarily light infections and do not exclude the possibility that exposure to helminths without patent infection boosts or maintains Ag-specific T cell responses, future studies should include measurements of the immune response to Ags expressed exclusively by infective-stage parasites (i.e., proteinases of *W. bancrofti*, third-stage larvae and *S. haematobium* cercariae). However, the fact that the majority of infants whose CBL responses indicated that they were not sensitized in utero continued to be nonresponsive when they are 10–14 mo old argues that the latter is unlikely. Third, comparison of the profile of cytokine responses at birth and 10–14 mo of age indicates that mixed as well as dominant type 2 responses persist in infants who are sensitized in utero. Longer periods of follow-up will be necessary to determine whether the allergic type 2 responses, which characterize Ag-specific responses by many adults, develop during early childhood. Finally, the data described here are in contrast to conclusions of other studies, which suggest that maternal microfilaremia leads to immunologic tolerance and predisposes to patent infection in children (20–22, 29). The reasons for this discrepancy may relate to the fact that none of the studies of older children (including the present one) ascertained maternal infection status during pregnancy, so that this variable may have differed from that assumed. It is also possible that the intensity of transmission during early childhood, independent of maternal infection status, has a major effect on the propensity to develop infection and microfilaremia later in life. Recent observations in Papua New Guinea and India indicate that quantification of spatial heterogeneity of transmission potential is important in such analyses (30–32).

The other major goal of the current study was to determine whether prenatal sensitization to filariae or schistosomes influences the immune response to BCG in newborns and children. With respect to 2–10-year-old children, there was a negative correlation between maternal infection with *W. bancrofti* and the ability of the child’s T cells to make IFN-γ in response to PPD. This diminution in PPD-driven type 1 responses was accompanied by increased generation of type 2 cytokines such as IL-5. The bias in T cell cytokine responses was limited to PPD and did not extend to the relationship between maternal filariasis and helminth Ag-specific T cell cytokine responses. Assuming that the infection status of the mothers was similar during the time of their pregnancy with the children under study, the data suggest that cytokine responses engendered by in utero sensitization to filariae affects the qualitative nature of T cell immunity to PPD for at least 2–10 years after BCG vaccination. Moreover, because filarial Ag-driven cytokine responses did not correlate with maternal infection, it is unlikely that the modulating effects of filarial infection acquired during childhood influence in vitro T cell immunity to PPD. We have not yet examined the interaction between filarial Ag- and PPD-driven cytokine responses in Kenyan adults, but a report of adults living in a filariasis endemic area of Indonesia showed that PPD-driven IFN-γ responses were not diminished among individuals who had strong type 2 (IL-4) responses to BmA (33).

Several caveats should be considered when drawing conclusions from cross-sectional studies such as those described above. Most importantly, this type of analysis does not ascertain directly whether an infant has been sensitized to helminth Ags in utero. In addition, worm infections acquired after BCG vaccination and repeated exposure to *M. tuberculosis* and other mycobacteria may influence PPD-specific immunity during childhood. Therefore, we determined the precise relationship between prenatal sensitization to helminths and immunity induced by BCG in a cohort of infants vaccinated on the day of delivery and examined at birth and 10–14 mo of age. Infants with documented prenatal sensitization to schistosomes or filarial Ags had diminished PPD-driven type 1 responses (IFN-γ and/or IL-2) and correspondingly increased type 2 responses (IL-4 and/or IL-5) relative to age-matched subjects who were not sensitized to the helminths at birth. The striking reduction in type 1 immunity in infants who were sensitized in utero was not attributable to infection with *W. bancrofti*, *S. haematobium*, or geohelminths acquired during the first year after birth. Moreover, T cell IFN-γ responses were most depressed in infants whose T cell responses at birth showed a dominant type 2 response.

The implication of these findings for the protective efficacy of BCG vaccination against tuberculosis remains to be determined. Because type 1 T cell responses, particularly IFN-γ, enhance the capacity of macrophages to kill *M. tuberculosis* and IFN-γ is involved in controlling mycobacterial infections (34–37), it will be informative to determine whether IFN-γ or other cytokines in culture supernatants of PPD-driven T cells from helminth-sensitized and nonsensitized infants differentially effect the ability of macrophages to eliminate mycobacteria in vitro. In the broader context of public health, the data are consistent with previous reports that adults with preexisting schistosomiasis, filariasis, or onchocerciasis have diminished reactions to BCG vaccination (38, 39). Because the incidence of clinical tuberculosis is low and difficult to measure accurately, especially in the tropics where this infectious disease is a major public health problem, studies of BCG efficacy in children will necessarily need to involve thousands of subjects. However, given the fact that BCG vaccination is a major strategy for limiting morbidity from tuberculosis in many areas of the world and control of worm infections such as bancroftian filariasis.
with mass chemotherapy may be feasible (40, 41), the design and implementation of such studies may be warranted.

References