Gamma Interferon Production in Response to *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* Antigens in Infants Born to Human Immunodeficiency Virus-Infected Mothers

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In utero sensitization to infectious pathogens can establish immunological memory and may influence the immune response to unrelated antigens. Little is known about the influence of intrauterine human immunodeficiency virus (HIV) exposure on the cellular immune response to mycobacterial antigens. Whole-blood culture gamma interferon (IFN-γ) production in response to mycobacterial antigens was measured at birth and 6 weeks of age to determine the characteristics of the IFN-γ response in HIV-exposed infants to *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* antigens. At birth, we observed an increased immune activation in response to phytohemagglutinin among HIV-exposed, uninfected infants. In a proportion of these infants, we also observed an increased immune activation in response to purified protein derivative, BCG, and early secreted antigen target 6. Increases in the IFN-γ response to the four antigens between birth and 6 weeks of age, observed in all HIV-unexposed infants, was absent in a substantial proportion of HIV-exposed, uninfected infants. The immunological differences persisted at 6 weeks of age, suggesting a sustained impact of in utero immune priming by HIV. Intrauterine exposure to HIV affects the infants’ cellular immune response to mycobacterial antigens, either specifically or as a consequence of nonspecific, broadly reactive immune activation. Further studies will be important to help determine optimal vaccination and disease prevention strategies for this vulnerable population group.

Every year, an estimated two million infants are exposed in utero to human immunodeficiency virus type 1 (HIV-1). Of these, 1.5 million are HIV uninfected and approximately 700,000 acquire HIV (2). At least 90% of these children live in sub-Saharan Africa.

Immunological changes associated with intrauterine HIV exposure have been described in HIV-uninfected infants born to HIV-infected mothers. HIV-specific cellular immune responses can be elicited among a proportion of these infants (11). Reduced interleukin-12 and interleukin-2 production in response to *Staphylococcus aureus* Cowan and phytohemagglutinin (PHA) stimulation suggests immunosuppressive effects of in utero HIV exposure (3, 16). An increase in activated (CD4+ HLA-DR+ CD38+) and memory (CD4+ CD45RA+ RO+) lymphocytes suggests in utero antigenic stimulation of CD4+ lymphocytes related to HIV products or antigens associated with opportunistic pathogens (16).

The immunological differences observed in HIV-exposed, uninfected infants are especially noteworthy given the immaturity of the neonatal cellular immune response (1). Immature, naive lymphocytes are the dominant lymphocyte phenotypes in infants (16). These naive cells are, compared to memory T cells, deficient in producing gamma interferon (IFN-γ) (5). Furthermore, neonatal T cells respond weakly to antigen-presenting cells, resulting in further decreased IFN-γ production (3, 19, 20). This low IFN-γ production may result in increased susceptibility to intracellular pathogens, including *Mycobacterium tuberculosis* (15).

In this study, we aimed to investigate the immune cytokine (IFN-γ) response to mycobacterial antigens and *Mycobacterium bovis* BCG vaccine among infants exposed to HIV in utero. In addition, we investigated the maturation of the cellular immune response of these infants in the first 6 weeks of life.

**MATERIALS AND METHODS**

**Study participants.** We enrolled 120 HIV-infected and 13 HIV-uninfected mothers and their infants between 19 January 2003 and 25 March 2003 at the Chris Hani Baragwanath Hospital in Soweto, South Africa. All of the women took part in the prevention of mother-to-child HIV transmission (PMTCT) program and had a known HIV infection status prior to enrollment. Infants with a birth weight of less than 2,500 g or with medical problems at birth were excluded. Clinical information, including HIV status, administration of nevirapine to mother and infant, history of active tuberculosis before and during pregnancy, and history of contact with active tuberculosis during pregnancy, was obtained with a structured questionnaire at the time of enrollment. Infants were followed up at 6, 10, and 14 weeks to collect data on feeding (exclusive breast-feeding, mixed feeding, or formula), occurrence of fever, development of a BCG scar (noted as papules, an ulcerating lesion, swollen axillary lymph nodes, and flat or healed), and contact with active tuberculosis.

Peripheral blood samples from the mother (at delivery) and infant (at day 1 and week 6) were collected in preservative-free Na-heparin Vacutainer tubes and
analyses were performed with SAS version 8.2 (Cary, NC, 2001) and SPSS 10.0 and Fisher's exact test to compare proportions between groups. All statistical response at birth of HIV-unexposed infants (group 1) or different from the response observed in HIV-infected infants was higher compared to their mothers'. The median infant log IFN-γ production in supernatants of whole-blood cultures stimulated with PHA, PPD, BCG, and ESAT-6 was significantly lower in infants at birth compared to their mothers'.

Comparisons of median IFN-γ production in PHA-, PPD-, BCG-, and ESAT-6-stimulated whole-blood cultures. The amount of in vitro IFN-γ production in supernatants was compared among four groups: infants born to HIV-infected mothers, infants born to HIV-uninfected mothers, HIV-infected mothers, and HIV-uninfected mothers. Unstimulated whole-blood samples (negative controls) produced undetectable or small amounts of IFN-γ. The median background unstimulated IFN-γ production was higher in HIV-infected or -exposed infants compared to HIV-uninfected mothers (P = 0.01); and 105.9 pg/ml and 55.9 pg/ml for HIV-exposed and HIV-unexposed infants at 6 weeks of age (P = 0.49).

RESULTS

One hundred thirty-three mother-infant pairs were enrolled. The first five mother-infant pairs were excluded from the analysis as their samples were used in the assay optimization process. Of the remaining 128 mother-infant pairs, 18 were excluded from the analysis because of loss of follow-up (seven mothers had relocated, three babies and one mother had died, two mothers withdrew, and five were untraceable). Four infants were HIV positive on PCR analysis at 6 weeks; no infants seroconverted between 6 and 14 weeks of age. The four HIV-infected infants and their mothers were also excluded from the analysis. Of the final 106 mother-infant pairs included in the analysis, 94 mothers were HIV infected and 12 mothers were HIV uninfected.

The level of immunosuppression among participating women was comparable to that described in other studies (6, 7): 12% had CD4 cell counts of <200, 14% had CD4 cell counts between 200 and 350, 27% had CD4 cell counts between 350 and 500, and 47% had CD4 cell counts of >500. Eighty-nine percent of the HIV-infected women and 90% of the HIV-exposed infants received nevirapine for PMTCT.

All of the infants enrolled in this study were vaccinated with BCG on day 1, and all developed a BCG scar, 96% by 6 weeks of age, 99% by 10 weeks of age, and 100% by 14 weeks of age.

Comparisons of median IFN-γ production in PHA-, PPD-, BCG-, and ESAT-6-stimulated whole-blood cultures. The amount of in vitro IFN-γ production in supernatants was compared among four groups: infants born to HIV-infected mothers, infants born to HIV-uninfected mothers, HIV-infected mothers, and HIV-uninfected mothers. Unstimulated whole-blood samples (negative controls) produced undetectable or small amounts of IFN-γ. The median background unstimulated IFN-γ production was higher in HIV-infected or -exposed infants compared to HIV-uninfected mothers and HIV-exposed infants. Unstimulated whole-blood samples (negative controls) produced undetectable or small amounts of IFN-γ. The median background unstimulated IFN-γ production was higher in HIV-infected or -exposed infants compared to HIV-uninfected mothers (P = 0.01); and 105.9 pg/ml and 55.9 pg/ml for HIV-exposed and HIV-unexposed infants at 6 weeks of age (P = 0.49). The median log IFN-γ production in supernatants of whole-blood cultures stimulated with PHA, PPD, BCG, and ESAT-6 was significantly lower in infants at birth compared to mothers and significantly higher in infants at 6 weeks of age compared to infants at birth (Fig. 1; Table 1). When we compared the immune responses of infants at 6 weeks of age and those of their mothers, we found that the median infant log IFN-γ production at 6 weeks was significantly lower for PHA (P < 0.01) whereas the response to BCG at 6 weeks of age among HIV-exposed infants was higher compared to their mothers' IFN-γ response to BCG (P < 0.0001) (Fig. 1; Table 1).

The median log IFN-γ production tended to be lower in HIV-infected compared to HIV-uninfected mothers and HIV-exposed compared to HIV-unexposed infants at 6 weeks of age. In contrast, the median IFN-γ production in response to BCG at birth was higher among HIV-exposed compared to HIV-unexposed infants (Fig. 1).

Correlation between maternal and infant IFN-γ production. Correlations between maternal and neonatal IFN-γ production were poor, with Spearman correlation coefficients (r values) of 0.13, 0.14, 0.19, and 0.27 for IFN-γ production in response to stimulation of whole-blood cultures with PPD, PHA, ESAT-6, and BCG, respectively. When investigating these correlations stratified by maternal HIV infection status, the r values were slightly higher for HIV-infected mothers and their HIV-exposed infants but correlations remained poor (r = 0.16, 0.25, 0.27, and 0.32 for PPD, ESAT-6, PHA, and BCG, respectively).

Poor correlations were also observed when comparing IFN-γ production between infants at birth and at 6 weeks of age (r values of 0.15, 0.07, 0.16, and 0.08 for PPD, PHA, ESAT-6, and BCG, respectively).

Intraindividual correlations between IFN-γ responses to PHA, PPD, ESAT-6, and BCG. In mothers, moderate correla-
tion coefficients were found between IFN-γ production in response to ESAT-6 and that in response to PPD ($r = 0.64, P < 0.001$, for HIV-infected mothers and $r = 0.56, P < 0.01$, for HIV-uninfected mothers) and between IFN-γ production in response to PPD and that in response to BCG ($r = 0.55, P < 0.001$, for HIV-infected mothers and $r = 0.40, P = 0.1$, for HIV-uninfected mothers). All other correlations were poor.

In infants at birth, the highest correlation coefficient was obtained between IFN-γ production in response to PPD and that in response to ESAT-6 ($r = 0.61, P < 0.001$). In infants at birth, the highest correlation coefficient was obtained between IFN-γ production in response to PPD and that in response to ESAT-6 ($r = 0.61, P < 0.001$).
6 weeks of age, the strongest correlation was observed between IFN-γ production in response to PPD and that in response to BCG (r = 0.76, P < 0.001), an indication of the immune response developed upon BCG vaccination.

Response to BCG vaccination and maturation of infant cytokine (IFN-γ) production. We observed that in HIV-unexposed infants, IFN-γ production in response to different antigens increased significantly from birth to 6 weeks of age, reflecting maturation of the cellular immune response (PHA) and immune response to BCG vaccination (PPD and BCG) (Fig. 2). Overall, IFN-γ production among HIV-exposed infants increased significantly for PPD and BCG and tended to increase for ESAT-6 and PHA. HIV-exposed infants could be categorized into two groups based on the pattern of change in the immune response from birth to 6 weeks of age. In one group (group 1), the change in IFN-γ production over time was similar to that of HIV-unexposed infants: an undetectable IFN-γ response at birth for PPD and ESAT-6 or a low IFN-γ response at birth (<0.33 log units for BCG and <2.3 log units for PHA), followed by an increase in the IFN-γ response at 6 weeks of age. The immune response in the other group of HIV-exposed infants (group 2) was characterized by a positive IFN-γ response at birth (≥2.3 log units for PHA, ≥0.33 log units for BCG, and >0 for PPD and ESAT-6) and a level of IFN-γ production at 6 weeks of age that was either moderately increased, similar to, or decreased relative to the amount of IFN-γ produced at birth (Fig. 2). The proportion of HIV-exposed infants belonging to one of these two groups was different for each of the stimuli investigated. The proportions of HIV-exposed infants classified in group 2 were 63, 45, 21, and 20% for the IFN-γ responses to PHA, BCG, ESAT-6, and PPD, respectively. While 32% of the infants were classified as group 2 for at least one of the four stimuli investigated (PHA, PPD, ESAT-6, or BCG), only 10% of the children were classified as group 2 for all four stimuli. The limited overlap of infants classified as having a group 2 response to different stimuli makes anergy to all stimuli in a subgroup of infants an unlikely explanation for the observed phenomenon.

We examined whether certain exposure variables may be responsible for the observed differential changes over time (birth to 6 weeks of age) in the infants’ ability to produce IFN-γ in response to PHA or mycobacterial antigens. No significant differences between the groups could be identified for the maternal CD4 count, a history of maternal exposure to active tuberculosis during pregnancy, maternal and infant exposure to nevirapine for PMTCT, or continued exposure to HIV through breastfeeding (Table 2).

**DISCUSSION**

Immunological memory can be established by in utero priming of T cells, and the immune environment established by in utero sensitization to specific antigens can influence immunity to unrelated antigens. In particular, Malhotra et al. demonstrated that cytokine responses engendered by in utero sensitization to filariae affects the cellular immune response to PHA (12). It has been suggested by Rich et al. that the increased immune activation documented in HIV-exposed, uninfected infants may be the result of in utero exposure to HIV antigens and/or exposure to antigens related to other infections in their HIV-infected mothers (16). In this study, we aimed to investigate the immune cytokine (IFN-γ) response to mycobacterial antigens and BCG vaccine among infants exposed to HIV in utero and the maturation of the cellular immune response of these infants in the first 6 weeks of life.

IFN-γ production in response to stimulation with PHA mitogen and mycobacterial antigens was significantly lower in infants at birth compared to that in mothers, supporting the well-established immature status of the neonatal immune response. Consistent with findings by Vekemans et al. (21), the median IFN-γ production in response to PPD and BCG increased significantly from birth to 6 weeks of age in both HIV-exposed and -unexposed children, indicating the establishment of an immune response following BCG vaccination. The median IFN-γ production in response to PHA increased significantly from birth to 6 weeks of age, indicating maturation of the infant’s immune response.

Similar to the observation described by Rich et al. (16), we observed an increased level of immune activation in response to PHA in a proportion of HIV-exposed infants at birth. In
FIG. 2. Changes in log IFN-γ production in stimulated cultures of whole blood from individual infants at birth (birth) and 6 weeks of age (6 weeks). Each pair of points connected by a line represents log IFN-γ levels for a single subject. For each stimulus investigated (PHA, PPD, ESAT-6, or BCG), the changes over time within each subject are presented for HIV-exposed and HIV-unexposed infants separately.
addition, we observed increased cytokine production in response to mycobacterial antigens in a proportion of HIV-exposed infants at birth, suggesting aspecific T-cell priming following in utero exposure to HIV. Even though the median IFN-γ production in response to PHA, PPD, and BCG was significantly higher at 6 weeks of age compared to that at birth in the group of HIV-exposed infants as a whole, a significant increase was absent in a substantial proportion (~32%) of the HIV-exposed, uninfected infants. This may indicate a lack of immune response to BCG, a lack of maturation of the cellular immune response, or a “fast-tracked” immune response in these infants so that their immune capacity at birth is comparable to that of a 6-week-old infant who has not been similarly primed in utero. These observations suggest that immunological differences observed at birth between HIV-exposed and HIV-unexposed infants may persist for at least the first 6 weeks of life.

We were especially interested in the neonatal and infant cellular immune response to BCG, a widely used tuberculosis vaccine in developing countries. As tuberculosis is the most important opportunistic pathogen in HIV-infected individuals, HIV-exposed infants are more likely than HIV-unexposed infants to be exposed to adults with active tuberculosis. A study in The Gambia has demonstrated that 2-month-old infants vaccinated with BCG at birth displayed strong proliferative responses and produced high levels of IFN-γ in response to PPD (13). Little information is available on the immunogenic and protective effects of BCG in HIV-exposed infants. In this study, we found that, in contrast to a report by Ota et al. (14), there was no difference between the proportions of HIV-exposed and -unexposed infants that develop a BCG scar, as 100% of the infants included developed a BCG scar by 14 weeks of age. There was also no significant difference in the median IFN-γ response to BCG between HIV-exposed and -unexposed infants at 6 weeks of age. In our study, the development of an immune response to the BCG vaccine was reflected by the strong correlation between IFN-γ production in response to PPD and BCG at 6 weeks of age, whereas at birth the strongest correlation was obtained between IFN-γ production in response to PPD and that in response to ESAT-6. An important difference between HIV-exposed and -unexposed infants was that in 45% of the HIV-exposed, uninfected infants, an IFN-γ response to BCG was already present at birth and did not increase subsequent to BCG vaccination. These findings may have important implications for the optimal timing and effectiveness of BCG vaccination in these infants and warrant careful immunological evaluation of new tuberculosis vaccines.

This study has several limitations. The short duration of follow-up of infants precluded the possibility of exploring the long-term effect of in utero exposure to HIV on the cellular immune response to mycobacterial antigens. The lack of data on intraindividual assay variability may have led to misclassification bias in some cases. The study could not determine the functional implication of the findings, i.e., the protective effect of BCG vaccination in HIV-exposed infants. The disadvantage of using a whole-blood assay was that we could not identify the cell types responsible for the increased IFN-γ production seen. While CD4+ lymphocytes are the main source of IFN-γ in response to PPD (21), up to 20% of IFN-γ-producing lymphocytes in the PPD-stimulated blood of infants and children are NK cells (8). We can therefore only hypothesize that the observed immune activation at birth in HIV-exposed, uninfected infants is due to aspecific T-cell priming following in utero HIV exposure. Several alternative explanations exist. HIV-infected women may have subclinical tuberculosis, leading to in utero exposure to mycobacterial antigens and development of a specific memory response in their infants (4, 18). Mycobacterium-specific responses, undetectable in the absence of exposure to HIV-1, could be “unmasked” in the presence of priming by HIV-1 in utero. Maturation stages of monocytes or other antigen-presenting cells could be different in these children. Non-specific priming could lead to IFN-γ production by cells other than T cells, such as NK cells (8). Cross-reactivity between HIV-1 and mycobacterial antigens may also have led to the observed immune activation (9).

Due to these study limitations, it remains unclear whether the higher IFN-γ response to mycobacterial antigens observed in HIV-exposed newborns represents nonspecific activation or in utero exposure to mycobacterial antigens. The study led to some interesting results that will allow hypothesis generation which can lead to investigations to further unravel the complexities. To gain further insight into the causal factors, determination of the mononuclear cell types and determination of

| TABLE 2. Comparison of exposure variables among HIV-exposed, uninfected infants whose cellular immune response, measured by in vitro IFN-γ production in response to PHA PPD, ESAT-6, and BCG, does (group 1) or does not (group 2) demonstrate a pattern of maturation between birth and 6 weeks of age |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Parameter       | PHAa            | PPDa            | ESAT-6a          | BCGa            |
|                 | Group 1         | Group 2         | Group 1         | Group 2         | Group 1         | Group 2         |
| No. of infants  | 35              | 59              | 75              | 19              | 74              | 20              | 52              | 42              |
| Mean maternal CD4 cell countb | 548              | 570              | 554              | 593              | 561              | 565              | 567              | 556              |
| Maternal CD4 cell count of <200 (%) | 17.7              | 8.6              | 9.5              | 22.2              | 11.1              | 15.0              | 15.4              | 9.5              |
| Exposure to active tuberculosis during pregnancy (%c) | 3.0              | 11.1              | 7.1              | 11.8              | 7.4              | 10.5              | 8.2              | 7.9              |
| Maternal nevirapine exposure (%) | 88.6              | 88.1              | 85.3              | 100              | 87.8              | 90.0              | 89.8              | 92.3              |
| Infant nevirapine exposure (%)d | 90.9              | 91.2              | 90.1              | 94.7              | 88.7              | 100              | 89.8              | 92.3              |
| Breastfeeding (%)e | 36.7              | 63.3              | 80.0              | 20.0              | 0.0              | 22.2e              | 54.4              | 45.6              |

a None of the differences were statistically significant by Fisher’s exact test.

b Missing information for two subjects.

c Missing information for seven mother-infant pairs.

d Missing information for four infants.
IFN-γ production from specific cell populations would be important. A negative control such as an antigen to which the fetus and infant are not exposed could be included to shed light on the specificity of the response to mycobacterial antigens. The inclusion of a control group of infants for whom BCG vaccination was delayed to 6 weeks of age would help differentiate responses due to mycobacterial exposure and BCG vaccination.

The qualitative differences in the immune response among a substantial proportion of HIV-exposed, uninfected infants demands further study of the early life immune response in these infants, its determining factors, and its clinical implications to help determine optimal vaccination and disease prevention strategies for this vulnerable population group.

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REFERENCES

1. Adkins, B. 1999. T cell function in newborn mice and humans. Immunol. Today 20:330–335.

2. Anonymous. 2004. 2004 report on the global AIDS epidemic: 4th global report. UNAIDS/04.16E. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland.

3. Choungnet, C., A. Kovacs, R. Baker, B. U. Mueller, N. L. Luhan, D. J. Liewehr, S. M. Steinberg, K. E. Thomas, and G. M. Shearer. 2000. Influence of human immunodeficiency virus-infected maternal environment on development of infant interleukin-12 production. J. Infect. Dis. 181:1590–1597.

4. Doherty, T. M., A. Demissie, J. Olobo, D. Wolday, S. Britton, T. Eguale, P. Ravn, and P. Andersen. 2002. Immune responses to the Mycobacterium tuberculosis-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients J. Clin. Microbiol. 40:704–706.

5. Ehlers, S., and K. Smith. 1991. Differentiation of T cell lymphokine gene expression: the in vitro acquisition of T cell memory. J. Exp. Med. 173:25–36.

6. Fawzi, W. W., G. I. Msamanga, D. Spiegelman, R. Wei, S. Kapiga, E. Villamor, D. Mwakagile, F. Mugusi, E. Hertzmark, M. Essex, and D. J. Hunter. 2004. A randomized trial of multivitamin supplements and HIV disease progression and mortality. N. Engl. J. Med. 351:23–26.

7. Guay, L. A., P. Musoke, T. Fleming, D. Bagenda, M. Allen, C. Nakabiito, J. Sherman, P. Bakaki, C. Ducar, M. Deseye, L. Emel, M. Mirochnick, M. G. Baltimore, D. Braven, L. Mofenson, P. Miotti, K. Dransfield, D. Bray, H. Miro, and J. N. Jackson. 1999. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda. HIVNET 012 randomized trial. Lancet 354:795–802.

8. Hanekom, W. A., J. Hughes, M. Masinkurwe, M. Mendillo, M. Watkins, H. Gamiendien, S. J. Gelderbloem, M. Sidibana, N. Manosoor, V. Davies, R. A. Murray, A. Hawrkiidge, P. A. Halsey, S. Ress, G. D. Hussey, and G. Kaplan. 2004. Novel application of a whole blood intracellular cytokine detection assay to quantify specific T-cell frequency in field studies. J. Immunol. Methods 291:185–195.

9. Hohn, H., C. Kortsik, G. Tully, K. Nilges, A. Necker, K. Freitag, C. Neukirch, P. Gaile, H. Lohr, and M. J. Mueeuer. 2003. Longitudinal analysis of Mycobacterium tuberculosis 19kDa antigen-specific T cells in patients with pulmonary tuberculosis: association with disease activity and cross-reactivity to a peptide from HIV env gp120. Eur. J. Immunol. 33:1613–1623.

10. Hussain, R., A. Kaleem, F. Shiahid, M. Duji, B. Jamil, H. Mehmoud, G. Dassou, and H. M. McAdam. 2004. IFN-γ- and TNF-α-producing mononuclear cells in peripheral blood can discriminate between tuberculosis patients and healthy endemic controls in a BCG-vaccinated population. J. Immunol. Methods 264:95–108.

11. Kuhn, L., S. Meddows-Taylor, G. Gray, and C. Tiemessen. 2002. Human immunodeficiency virus (HIV)-specific cellular immune responses in newborns exposed to HIV in utero. Clin. Infect. Dis. 34:267–276.

12. Malhotra, L., P. Mungai, A. Wamacli, J. Kioko, J. H. Ouma, W. J. Kazura, and C. L. King. 1999. Helminth- and bacillus Calmette-Guérin-induced immunity in children sensitized in utero toilaris and schistosomiasis. J. Immunol. 162:6843–6848.

13. Marchant, A., T. Geothgeber, M. O. Ota, I. Wolfe, S. J. Ceesay, D. De Groote, T. Corrah, S. Bennett, J. Wheeler, K. Huysgen, P. Aaby, K. P. McAdam, and M. J. Newport. 1999. Newborns develop a Th1-type immune response to Mycobacterium bovis bacillus Calmette-Guérin vaccination. J. Immunol. 163:2249–2255.

14. Ota, M. O., D. O’Donovan, A. Marchant, L. Yamuah, E. Harding, S. Jaffar, K. P. McAdam, T. Corrah, and H. Whittle. 1999. HIV negative infants born to HIV-1 but not HIV-2 positive mothers fail to develop a bacillus Calmette-Guérin skin. AIDS 13:996–998.

15. Ottenhof, T. H. M., D. Kumararatne, and J. L. Casanova. 1998. Novel immunodifficulties reveal the essential role of type-1 cytokines in immunity to intracellular bacteria. Immunol. Today 19:491–495.

16. Rich, K., J. N. Siegel, C. Jennings, R. J. Rydman, and A. L. Landay. 1997. Function and phenotype of immature CD4+ lymphocytes in healthy infants and early lymphocyte activation in uninfected infants of human immunodefiency virus-infected mothers. Clin. Diagn. Lab. Immunol. 4:358–361.

17. Scholvinck, E., K. A. Wilkinson, A. O. Whelan, A. R. Martineau, M. Levin, and R. J. Wilkinson. 2004. Gamma interferon-based immunodiagnosis of tuberculosis: comparison between whole-blood and enzyme-linked immuno-spot methods. J. Clin. Microbiol. 42:829–831.

18. Singh, K. K., Y. Dong, J. T. Belisle, J. Harder, V. K. Aarora, and S. Laal. 1999. Antigens of Mycobacterium tuberculosis recognized by antibodies during incipient, subclinical tuberculosis. Clin. Diagn. Lab. Immunol. 6:354–355.

19. Suen, Y., S. M. Lee, J. Qiam, C. Van de Ven, and M. S. Cairo. 1998. Dysregulation of lymphokine production in the neonate and its impact on neonatal cell mediated immunity. Vaccine 16:1363–1368.

20. Trivedi, H. N., K. T. HayGlass, V. Gangur, J. G. Allardice, J. E. Embree, and F. A. Plummer. 1997. Analysis of neonatal T cell presenting cell function. Hum. Immunol. 57:69–79.

21. Vekemans, J., A. Amelde, M. O. Ota, M. D’Elios, T. Geothgeber, J. Ismail, M. J. Newport, G. Del Prete, M. Goldman, K. P. McAdam, and A. Marchant. 2001. Neonatal bacillus Calmette-Guérin vaccination induces adult-like IFN-γ production by CD4 lymphocytes. Eur. J. Immunol. 31:1531–1535.