T cell responses to vaccines in infants: defective IFNγ production after oral polio vaccination

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SUMMARY

The immaturity of the neonatal immune system is associated with an increased susceptibility to infections. Studies in mice indicate that neonatal immune responses are biased towards the T helper 2 type, but little is known about helper T cell responses in human newborns. In this study, the oral polio vaccine was used as a model of early immunization to investigate the capacity of young infants to develop cellular immune responses. We show that neonatal immunization with oral polio vaccine induces the production of high titres of neutralizing antibodies but reduced proliferative and IFNγ responses to polio antigens compared to immune adults. These data suggest that specific strategies will be required to immunize newborns against pathogens controlled by Th1 type immune responses.

Keywords cellular immunity infant poliomyelitis vaccination

INTRODUCTION

Neonatal immunization is required to protect young infants against early infectious pathogens, but is impaired by the immaturity of the immune system [1]. Experiments performed in mice indicate that neonatal immune responses to vaccines are defective in the T helper 1 (Th1) type and biased towards Th2 type responses [2–4]. Th1 lymphocytes are specialized in the production of IFNγ and are essential to the control of intracellular pathogens, whereas Th2 lymphocytes produce interleukin (IL)-4, IL-5 and IL-13, support antibody production and are involved in allergic disorders.

Relatively little is known about human helper T cell responses in early life [1,5]. Impairment in Th1 responses in human infants could reduce the efficacy of vaccines against intracellular pathogens such as the respiratory syncytial virus, measles, malaria and the human immunodeficiency virus [1]. Recent data showing that human cord blood-derived dendritic cells have a profound defect in the production of IL-12 suggest that Th1 responses could also be defective in human newborns [6]. However, we have reported previously that neonatal *Mycobacterium bovis* Bacillus–Calmette–Guérin (BCG) vaccination induces a Th1 type response similar to that measured in immune adults [7,8].

The oral polio vaccine (OPV) is another vaccine given to newborns in developing countries as part of the strategy to eradicate poliomyelitis. Immunization with multiple doses of OPV in early life induces the production of large amounts of neutralizing antibodies that are essential to protection against poliomyelitis [9]. The T cell response to OPV has not been characterized in detail. This study was undertaken to characterize further helper T cell responses to vaccines in young infants, using OPV as a model of early immunization. T cell proliferation as well as Th1 and Th2 cytokine production induced by polio antigens were measured in infants vaccinated at birth, 1, 2 and 3 months of age and compared to that of immune adults following booster immunization.

MATERIALS AND METHODS

Study subjects

This study was approved by the Gambia Government/MRC joint Ethics Committee. Babies were enrolled at birth at Serrekunda Health Centre after maternal consent. A sample of cord blood was collected. The infants were vaccinated as recommended by the National Immunization Programme, including a dose of OPV (Sabin, Glaxo SmithKline, Rixensart, Belgium) at birth and at the age of 1, 2 and 3 months. Other vaccines were delivered according to the national programme of immunization. A blood sample was collected from 27 infants 3 weeks after the last dose of OPV. Thirty-six adult volunteers (age range: 15–20 years) were recruited from a population living in the rural area of Keneba that has been under epidemiological surveillance by the MRC for over 50 years. According to our records, all these individuals had received at least three doses of OPV in infancy. For the purpose

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of the study, they were given a booster dose of OPV and were then bled 3 weeks later.

Preparation of polio virus antigens
Poliovirus Sabin types 1, 2 and 3 (NIBSC, Hertfordshire, UK), at a stock concentration of 7 $TCID_{50}$/0·1 ml were diluted 1 : 700 in MEM media supplemented with penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM) (all from GIBCO Laboratories, Grand Island, NY, USA), and 12 ml were used to seed each of eight confluent T175 flasks of HEp2C cells (European Collection of Cell Cultures, Salisbury, UK). Cytopathic effect was observed after 12–24 h of incubation at 37°C. Virus-containing supernatant was harvested, frozen and thawed three times, heat inactivated at 56°C for 1 h and frozen for storage. Supernatant from noninfected cells was used as control.

Cellular immune responses to polio antigens
PBMC were isolated from fresh heparinized blood samples from vaccinated infants and adults by density gradient centrifugation. Cells were resuspended in cell culture medium containing 10% heat-inactivated pooled human AB serum (Sigma Chemicals, St Louis, MO, USA). Preliminary experiments indicated the optimal antigen concentrations and incubation periods to induce proliferative and cytokine responses (data not shown). Then 200 000 cells/200 µl medium were incubated in quadruplicates in the presence of PHA, PV1, PV2 and PV3 antigens (concentration of 1 : 8, vol : vol), PHA-L (5 µg/ml, Sigma Chemicals) or medium alone in U-bottomed 96-well plates at 37°C, 5% CO_2. Supernatants were collected on day 2 from PHA-stimulated wells and on day 6 from unstimulated and polio-stimulated wells. On day 6, 1 µCi [methyl-^{3}H] tritiated thymidine per well was added to the cell cultures for an additional 17 h to assess cell proliferation. Thymidine incorporation was measured by liquid scintillation using a Betaplate reader (LKB1205, Turku, Finland). Commercially available ELISA kits were used to determine cytokine concentrations in supernatants (IFNγ and IL-5: Biosource Europe, Fleurus, Belgium; IL-13: Diaclone, Besançon, France). IFNγ, IL-5 and IL-13 detection limits were 8 pg/ml.

Frequencies of IFNγ-producing lymphocytes
The frequency of IFNγ-producing lymphocytes was determined by ELISPOT using commercially available reagents according to the recommendations of the manufacturer (Mabtech, Stockholm, Sweden). 2×10^5 PBMC/well were incubated in 96-well ELISPOT plates (MAIPS45, Millipore, Bedford, MA, USA) in quadriplicate in the presence of PV1, PV2, PV3 antigens or PHA for 16 h. The numbers of spot-forming cells were counted using an automated Elispot reader (AID Autoimmun Diagnostic GmbH, Strassberg, Germany).

Anti-polio virus neutralizing antibody concentrations
Serum neutralizing antibodies to poliovirus types 1, 2 and 3 were measured according to the methodology recommended by the World Health Organization [10]. Briefly, sera were heat-inactivated and serial twofold dilutions prepared in a microtitre-format plate. Standard volumes of poliovirus types at 100 $TCID_{50}$ were added to the diluted sera, and the plate incubated at 37°C for 3 h in 5% CO_2. Next, 1–2 × 10^4 HEp-2 cells/well were added. The HEp-2 cell line is particularly sensitive to polioviruses. The plate was further incubated for 6 days at 37°C in 5% CO_2 and the monolayers were assessed for viral cytopathic effect. The serum antibody titre was reported as the highest dilution of serum that protected 50% of the cultures against virus challenge, expressed as the reciprocal of the titre, and converted into international units.

Statistics
Comparisons of serum neutralizing antibody levels were done on log-transformed data using the *t*-test on paired (comparison of cord blood and infant sera) and unpaired data (comparison of adult and infant sera). Proliferation results were calculated as stimulation indexes (SI, counts in wells stimulated with polio-infected HEp-2 supernatant/counts in wells stimulated with uninfected HEp-2 supernatants), log-transformed and compared using the *t*-test. A positive proliferative response was defined as a SI of more than 3. Cytokine concentrations and IFNγ producing lymphocyte frequencies were compared using the Mann–Whitney–Wilcoxon test. A positive cytokine response was defined as a concentration in antigen-stimulated wells above twice the concentration measured in wells containing the control supernatant from non-infected cell lines.

RESULTS

As shown in Fig. 1, low concentrations of maternal anti-PV1, -PV2 and -PV3 antibodies were detected in cord serum. The administration of four doses of OPV at birth, 1, 2 and 3 months of age induced the production of large concentrations of anti -PV1, -PV2 and -PV3 neutralizing antibodies. The antibody concentrations measured in infants were higher than that measured in immune adults following a booster OPV immunization, although the difference did not reach statistical significance in the case of anti-PV3 antibodies.

In keeping with previously reported data [11], incubation of peripheral blood mononuclear cells (PBMC) in the presence of PV1, 2 and 3 antigens induced a proliferative response in 36, 5 and 30 of the 36 adults studied, respectively (Fig. 2a). In contrast, proliferative responses to PV 1, 2 and 3 were detected in only 5, 0 and 3 of the 27 infants studied, respectively. Median proliferative responses to the three viral preparations were significantly
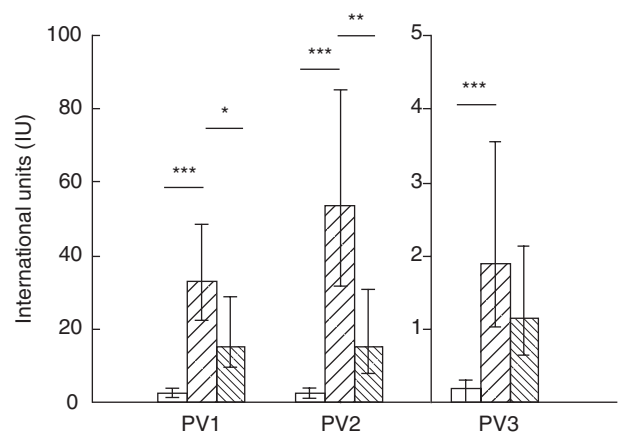
Fig. 1. Anti-PV1, 2 and 3 neutralizing antibody concentrations (international units/ml) in serum from cord blood, vaccinated infants and immune adults. Geometric means and 95% CI are presented. *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$. ▫, Cord serum; ▨, infant serum; ◩, adult serum.
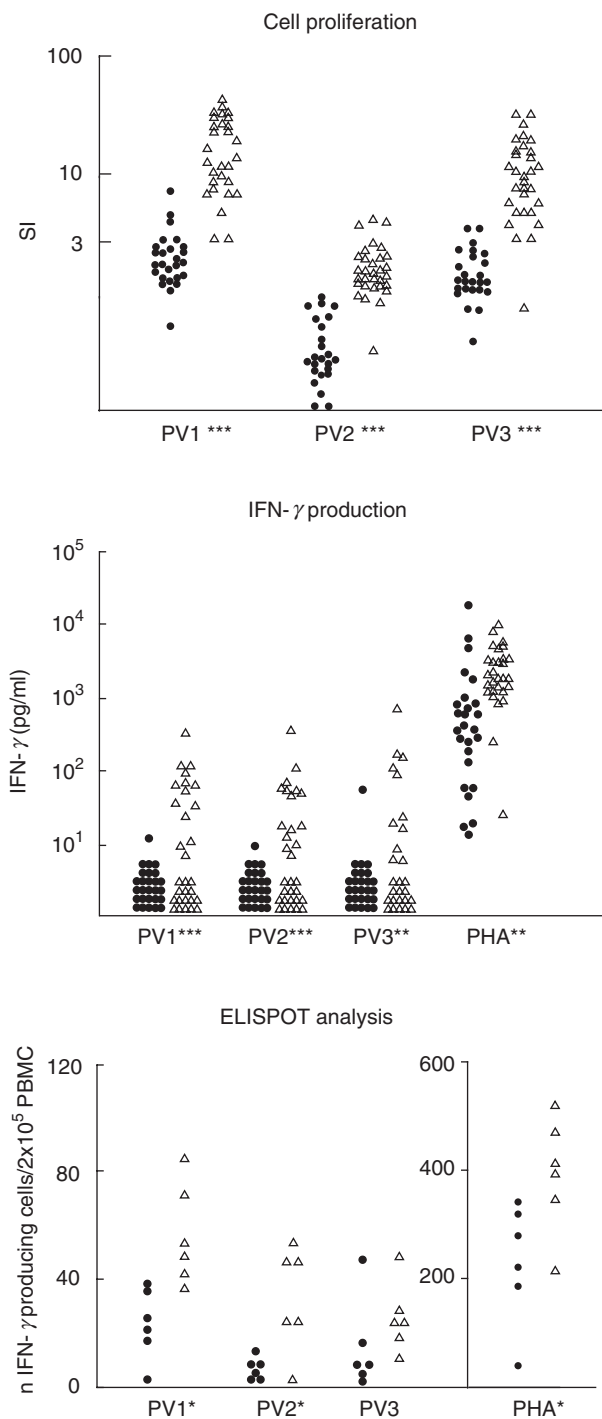
Fig. 2. Cellular responses to PV1, 2 and 3 antigens in vaccinated infants (●) and immune adults (△). Individual values are shown. a, proliferative responses. Stimulation indexes (counts in wells stimulated with polio antigen-containing supernatant/counts in wells stimulated with control supernatant) are presented. b, IFNγ concentrations (pg/ml) in supernatants from antigen-stimulated cell cultures. c, number of IFNγ-producing cells/200×10^5 PBMC. *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$.

lower in infants than in adults, whereas background proliferation values were similar in both groups (data not shown). In adults, the strong proliferative responses to PV1, PV2 and PV3 antigens were associated with a detectable IFNγ production in supernatants in 14, 15 and 11 of 36 adults, respectively (Fig. 2b). In contrast, only one, none and one of the 27 infants produced detectable concentrations of IFNγ in response to PV 1, 2 and 3 antigens, respectively. Median IFNγ responses to the three polio antigen preparations were significantly lower in infants than in adults. Infants also produced lower concentrations of IFNγ in response to PHA, as reported previously [5]. Both infants and adults produced high concentrations of IL-5 and IL-13 in response to PHA stimulation but no IL-5 or IL-13 could be detected in response to stimulation with polio antigens (data not shown). The number of IFNγ producing lymphocytes in response to polio antigens was studied in six adults and six infants using the ELISPOT technique (Fig. 2c). Infants showed detectable but significantly lower frequencies of IFNγ producing lymphocytes following stimulation with PV1 and PV2 antigens compared to adults.

DISCUSSION

In this study, we show that young infants have defective IFNγ response to OPV, compared to re-immunized adults. Adults enrolled in this study had been vaccinated with OPV in infancy and had probably been exposed to the virus shed from vaccinated infants in their environment. The defective IFNγ, response to OPV in infants is not likely to be due to reduced exposure to the vaccine, as they had higher titres of neutralizing antibodies compared to adults. These antibodies were not derived from the mother, as cord sera contained only low levels of antipolio antibodies. The difference in antibody concentrations in adults and infants indicates that the administration of multiple doses of OPV in naive infants induces a higher antibody response than the administration of a single dose of OPV in previously immune individuals. In humans, while T cell response to poliovirus may play a role in limiting viral replication and disease, protection against poliomyelitis infection is dependent on the induction of an antibody response [9]. Therefore, our observations do not challenge the recommendations of WHO to immunize newborns in developing countries [9].

This study demonstrates for the first time a defective production of IFNγ in response to a vaccine in early infancy. This defect may be involved in the increased susceptibility of infants to intracellular pathogens. Indeed, severe herpes and respiratory syncitial virus infections in infants are associated with reduced or delayed IFNγ responses [12,13]. The reduced IFNγ response to OPV in infants contrasts with the adult-like IFNγ response induced at birth by BCG [8]. Although the high proportion of recent thymic emigrants could be involved, the immaturity of neonatal antigen-presenting cells is likely to play an important role [14]. BCG is a potent activator of adult dendritic cells, increasing their production of IL-12 and their expression of costimulatory molecules and could overcome the defective costimulatory properties of neonatal dendritic cells [6,15]. A similar mechanism is likely to play a role in the different responses induced by whole cell or acellular pertussis vaccines in infants. Whole cell pertussis induces the production of IL-12 by macrophages and induces an IFNγ dominant response in infants. In contrast, infants produce both Th1 and Th2 cytokines in

response to immunization with the acellular pertussis vaccine [16–18].

Th2 cytokines were not detected in response to OPV antigens in infants and adults. The IFNγ dominant response to OPV observed in adults is in keeping with results obtained in mice transgenic for the human poliovirus receptor [19]. In this experimental system, OPV induced a Th1 response that provided help to B lymphocytes and protected animals against a lethal poliovirus infection. The origin of T-cell help to high antibody production by B cells in infants with little Th1 or Th2 cytokine production remains unclear. Adkins *et al.* [4] reported that Th2 type responses are preferentially induced in the spleen of newborn mice. As we measured cytokine responses in peripheral blood, we cannot rule out the possibility that OPV vaccinated infants had increased Th2 cytokine production in the spleen. Nevertheless, in contrast to what is observed in mice [2–4], this study suggests that early life vaccination in humans does not systematically induce Th2-dominant responses.

In conclusion, our study shows that young infants have a defective IFNγ response to OPV. Defective IFNγ production could play an important role in the susceptibility of young infants to intracellular pathogens. In newborn mice, adult-like Th1 type responses can be induced by the administration of Th1-promoting adjuvants, IL-12, CpG-containing oligonucleotides, DNA vaccines or replicating viruses [1,5]. Protection of human infants against pathogens controlled by Th1 responses will probably require specific strategies inducing the activation of neonatal antigen-presenting cells.

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