Published in final edited form as:
Vaccine. 2014 June 30; 32(31): 3886–3894. doi:10.1016/j.vaccine.2014.05.008.

Challenges in Vaccination of Neonates, Infants and Young Children

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Abstract

All neonates, infants and young children receive multiple priming doses and booster vaccinations in the 1st and 2nd year of life to prevent infections by viral and bacterial pathogens. Despite high vaccine compliance, outbreaks of vaccine-preventable infections are occurring worldwide. These data strongly argue for an improved understanding of the immune responses of neonates, infants and young children to vaccine antigens and further study of the exploitable mechanisms to achieve more robust and prolonged immunity with fewer primary and booster vaccinations in the pediatric population. This review will focus on our recent work involving infant and young child immunity following routine recommended vaccinations. The discussion will address vaccine responses with respect to four areas: (1) systemic antibody responses, (2) memory B-cell generation, (3) CD4 T-cell responses, and (4) APC function.

Keywords

Vaccination; Neonate; Infants; Children; B cells; T cells; Antigen presenting cells; Dendritic cells; immunologic memory; pediatric vaccines; *Streptococcus pneumoniae*; *Haemophilus influenzae*; B-cell receptor; MHC II; CD4 T-cells; cytokines; T-cell receptor; Toll-like receptor

Introduction

One of the biggest challenges facing vaccinations is that 19.3 million infants and children throughout the world do not receive the multiple recommended doses of vaccines required to achieve optimal immunity [1–6]. Aside from the many issues facing worldwide vaccination programs, there are environmental and genetic factors that affect the development of the immune system that also contribute to high mortality especially in neonates, infants and young children[3]. We are focusing on the mechanisms involved in the vaccine responses in these youngest of pediatric populations. This review will mainly focus on the work being done in our lab to address this important issue.

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Maternal antibody and poor generation of T-cell and B-cell memory in neonates and infants are known to result in inadequate adaptive immunity from vaccinating this population compared to older children and adults [7–20]. Our group has recently identified a subset of infants and young children that fail to generate protective antibody levels to diphtheria (DT), tetanus (TT), pertussis (PT) toxoid, pertussis filamentous hemagglutinin (FHA), and pertussis pertactin (PRN) in DTaP vaccinations, polio serotype 3, and *Streptococcus pneumoniae* conjugated polysaccharide 23F (Prevnar-CRM) and produce lower geometric mean titers to polio serotypes 1 and 2 and, *Streptococcus pneumoniae* serotype 14 [21]. However, we did not observe an increase incidence of infections caused by diphtheria, pertussis, tetanus, etc. and reasoned that this could be due to limited-exposure and/or herd immunity. Therefore, we elected to study seasonal influenza infections since they occur as widespread annual community-wide outbreaks. We found that otitis prone, OP, children show inadequate immune responses to influenza vaccination and therefore 10-fold more influenza infections (Verhoeven et al, Vaccine 2014, submitted for publication). These same children have $CD4^+$ T-cell memory recall responses to PT, FHA and PRN that are significantly inferior in quality as compared to adult responses[22]. We are calling these children “low vaccine responders” (LVR), as compared to “normal vaccine responders” (NVR), and have observed that they have features resembling a neonate’s immune system[21–26].

We serendipitously discovered this group of low vaccine responders during our work involving infants and young children prone to recurrent middle ear infections [27–30]. In that research we identified a cohort of young children, 15 (5.9%) of 254, that experienced frequent recurrent middle ear infections, despite individualized care that included tympanocentesis drainage of acute otitis media (AOM) episodes and modification of antibiotic therapy as needed according to the otopathogen isolated and its antibiotic susceptibility [31]. We called these children stringent otitis prone (sOP) due to the stringent requirement of tympanocentesis-proven middle ear infections. Subsequently, we now have over 40 children out of 700 in our prospective study cohort who meet the sOP criteria. We hypothesized and showed that the propensity to recurrent AOM could be attributed to poor adaptive immune responses following infection by the dominant otopathogens *Streptococcus pneumoniae* and *Haemophilus influenzae*. Specifically we found low or absent antibody and cellular responses to vaccine candidate antigens PhtD, PhtE, Ply and LytB but less so to PcpA of *Streptococcus pneumoniae* [24, 27] and to protein D and OMP26 but less so to P6 of *Haemophilus influenzae* [28, 29]. Also, the children exhibited poor antigen-specific memory T-cell responses to *Streptococcus pneumoniae* and *Haemophilus influenzae* antigens, although they responded normally to Staphylococcal enterotoxin B, suggesting the primary immune defect might involve multiple factors such as poor antigen presenting cell (APC) function, altered innate responses or lower toll-like receptor expression [22, 23, 26, 32, 33].

Display of similar immune dysfunction in neonates, infants and young children following vaccination suggests the possibility of involvement of common cell types and mechanisms. Through studying dynamic differences in immune responses over time a better understanding of the state of flux of the immune response should be attainable as neonates

and infants rapidly mature from the neonatal regulated state to a metered inflammatory phenotype to protect from disease but limit immunopathology.

Systemic antibody responses

Vaccination produces protective benefits primarily by induction of systemic antibodies [34–38]. Neonates, infants and young children produce lower vaccine-specific IgG serum titers than older children or adults to most vaccines[39].

In Figure 1 changes in pediatric vaccine antibody titers over time for 68 age-matched infants and young children from age 6 to 30 months is shown. LVRs (red) selected from a cohort of sOP children and normal vaccine responders (black) selected from a cohort of non-otitis prone children are shown. The nadir of low titers at age 9–15 months old is seen, with improvement after first boosters (measured at 24 months), varying among vaccines. From the results we established an operational classification of children as normal vaccine responder when protective antibody levels to >80% of recommended vaccine antigens tested is achieved. A LVR would be an infant/child with below protective antibody titers to >50% of recommended vaccines tested [40].

We have also analyzed differences in immune response to influenza vaccination and occurrence of infection in LVRs. In that study we found plasma IgG responses to purified hemagglutinin HA1 or HA3 did not correlate with failure to protect against influenza infection. Instead it was the quality of the antibody as determined by hemagglutination inhibition titers and viral neutralizing antibody titers that identified bona fide LVRs who more frequently contracted influenza infection (Verhoeven et al Vaccine 2014, submitted We have also studied immune responses to RSV. sOP children who are LVR, experience higher RSV viral burdens, lower RSV-specific IgG and neutralizing antibody levels that correlate with diminished T-cell responses to RSV. (Verhoeven et al Clin Inf Dis 2014, revision submitted). In addition, these LVR children infected with RSV show lower expression of TLR7 on isolated APCs and lower level of activated HLA-DR expression on B-cells infected with RSV.

Memory B-cell generation

The ability of B-cells to proliferate and differentiate into memory and plasma cells influences the levels of protective antibodies. Infants and young children can elicit adult-like antibody avidity profiles after early-life immunization with protein vaccines[41]. But the underdeveloped B-cell repertoire and the absence of previous antigenic exposure leads to lower level of protective antibody [42].

We have recently studied antigen-specific memory B-cells [24] to provide a more precise understanding of dysfunctional mechanism(s) leading to reduced B-cell maturation to IgG-secreting plasma cells in infants and young children. We found that B-cell frequencies in the peripheral circulation correlated with serum levels of antigen-specific Ig responses of sOP infants and young children who were LVRs (Fig. 2) resembled B-cells of neonates. Immaturity in the neonatal B-cell repertoire may include a reduced strength of B-cell

receptor (BCR) signaling, under-expressed co-stimulatory receptors and lower activation signals [43, 44].

Two mechanisms in B cells likely account for much of the immune dysfunction in neonatal, infant and young children: inadequate B-cell receptor (BCR) signaling and lower levels of MHC class II (MHC II) expression (Fig. 3). CD22 is a surface exposed molecule that affects apoptosis and BCR signaling[44, 45]. Neonatal B cells or cord blood lymphocytes show differential expression of CD22 depending upon antigen stimulation compared to adult B cells, resulting in either apoptosis or impaired B cell activation and differentiation[44]. Expression of MHC II molecules and their ability to present processed antigenic peptides to T helper cells play an important role in B-cell activation, proliferation, Ig isotype switching and somatic hypermutation[46, 47]. Neonatal B-cells express lower levels of MHC II are less effective in antigen processing and presentation to T-cells. CD40 is another important receptor on B cells that interact with CD154 (CD40L), a co-stimulatory molecule on T cells that also regulates B-cell function[48]. The neonatal immune system has showed an immaturity at CD40-CD40L in the T-cell interaction with B cells and monocytes[49]. Lower levels of CD154 on T-cells also results in lower expression of signaling cascade proteins and lower expression of cytokine genes[50].

Follicular T-helper, Tfh, cell is another T-helper subset to help B cells differentiate into plasma cells and memory cells[51]. We are interested in the B-cell memory percentages in normal and low vaccine responding infants and whether peripheral blood levels of Tfh cells may reflect the vaccine responses. Preliminary data in our laboratory suggests that circulating blood levels of TFH cells in 6-month and 12-month old infants are significantly lower compared to older children and adults. We also found that the quantity of TFH cells in tonsils increases with age, as would be expected with “outgrowing” the immunological delay of CD4 T-cell activation (unpublished results). Importantly, lower percentages of TFH cells in tonsils of children who are LVRs were measured compared to age-matched normal vaccine responders (data not shown).

CD4 T-cell responses

Although there are many different T-cell cell types, CD8 and CD4 are the main T-cells in adaptive immunity responsible for T-cell killing of infected cells and B-cell help. Function of CD8 T-cells among neonates, infants and young children is similar to older children and adults[52]. However, there are phenotypic changes of CD8 T-cells depending on the vaccine and the age of the person[53] along with neonates, infants and young children having defective DC-induced IL-12 secretion[54].

CD4 T-cells can be broadly divided into different subsets based on their cytokine secretion and function[55–59]. CD4 T-cells that produce mainly IFN-γ, TNFα and IL-2 are designated Th-1 while those that produce predominantly IL-4, IL-5, and IL-10 are designated Th-2. Neonates, infants and young children polarize their vaccine-mediated CD4 T-cell responses to Th-2 whereas adults have a more balanced Th-1/Th-2 response.[3, 60–63] Differences in CD4 T-cell responses to vaccination could dramatically affect the quality of B-cell responses after vaccination[39, 64, 65]. For instance, low vaccine responses in

neonates, infants and young children are thought to be due to CD4 T-cell dysfunction [22–24, 66]. Their CD4 T-cells may fail to stimulate antibody-secreting B-cells because of intrinsic T cell-related mechanisms and/or extrinsic effects of deficient APC function[16, 23, 25, 67] failing to properly stimulate naïve CD4 T-cells [66].

The neonatal T-cell response is marked by poor IFN-γ production, and vaccine-specific responses characterized by a generalized bias towards IL-4 production[3, 68]. We have recently determined that CD4 T-cell responses in normal infants and young children after *Staphylococcal* enterotoxin B (SEB) mitogenic stimulation exhibit lower polyfunctional cytokine responses as compared to adults (Fig. 4D)[25] and pattern an immunological maturation with age with respect to increasing IFN-γ production (data not shown).

To assess the differences in T-cell responses to acellular pertussis vaccination in terms of quality and quantity of the response persisting after vaccination, we stimulated peripheral blood mononuclear cells (PBMCs) from infants, children and adults who were recently vaccinated with DTaP or Tdap, respectively and found lower polyfunctional responses in infants and children compared to adults (Fig. 4A–C)[25]. We also observed lower TNFα/IFN-γ responses to acellular pertussis antigens and SEB in infants (data not shown) suggesting that the strength of the priming TCR signal or homeostatic maintenance of memory subsets after polarization may impact the quality of the recall response. Our results demonstrate a divergence in circulating T-cell memory percentages for vaccine antigens in infants and children compared to adults[25].

Epigenetic modulation of effector cytokines may inhibit CD4 T-cell responses in neonates but whether this persists into early childhood is not known [69, 70]. Low IFN-γ or IL-4 antigen specific recall responses to vaccine antigens persist until at least 1 year of age even with in vitro APC supplementation, suggesting that intrinsic blocks to cytokine responses such as epigenetic regulation of pro-inflammatory responses may persist past the neonatal stage[17]. We have shown that the percentages of $CD69^+$ CD4 T-cells in recall assays to be similar in infants and children compared to adults suggesting that differences in APC stimulatory function may not fully account for diminished normal CD4 T-cell responses in infants and children[25]. The data suggest that CD4 T-cells in infants and children could have intrinsic blocks to robust IFN-γ or IL-4 secretion as compared to older children or adults and we seek to better understand the role of diminished production of these effector cytokines as they relate to regulating vaccine specific memory quality.

Compared to adult CD4 T-cells, neonatal CD4 T-cells show reduced expression of several transcription factors including signal transducers and activators of transcription factor 4 (STAT4), t-bet, and c-maf during primary stimulation and are less responsive to antigen presentation[50]. Three mechanisms likely predominate to explain the low vaccine-specific memory CD4 T-cells in neonates, infants and young children: 1) Impaired TCR stimulation; 2) Increased apoptosis after proliferation; and 3) Compromised functionality of generated effector and/or memory cells (Fig 5).

Impairment of CD4 T-cell activation could be due to a defect in the TCR signaling pathway that activates protein phosphorylation and activation of phospholipases[71]. Experiments

with umbilical cord blood T-cells have shown that antigen stimulation does not influence TCR-CD3 surface expression, but initializes TCR internalization and downstream signaling including activation of phospholipase C-γ (PLC) by Src family kinases, Syk, Lck, ZAP70 and Fyn[72]. The low expression of PLC and Syc, Lck and Fyn constitute molecular defects in the signaling pathway that activates the promoters of various cytokines including IL-2[73].

Previous studies have demonstrated that naïve neonatal CD4 T cells also undergo apoptosis in response to primary TCR-mediated stimulation and also after activation with anti-CD3[74]. This was observed due to the high caspase-mediated cell death as a control mechanism of antigen-independent expansion by cord blood RTEs [75]. However, under appropriate stimulatory conditions and stimulating with anti-CD3/anti-CD28, naïve umbilical cord blood CD4 T-cells can generate competent Th effector cells [17]. IL-12, IFN-γ and IL-4 induce differentiation in newly activated naïve CD4 T-cells and help to stabilize the subset polarity through enforcement of either T-bet or Gata-3 expression[76]. We have data from infants and children that shows low IFN-γ production upon stimulation of memory CD4 T-cells with vaccine antigens[25]. This could be due to an impaired ability to differentiate in response to differentiating signals from cytokines and/or DCs.

Experiments with cord blood cells has shown that neonates have very low frequency Th17 cells due to their intrinsic defect of downstream signaling of transcription factor RORC (Retinoid acid–related Orphan Receptor C)[77]. We have observed delayed generation of IL-17 responses in PBMCs in infants and children to bacterial, vaccine antigens, and SEB stimulation as compared to older children and adults, suggesting differential regulation of this T-cell subtype. Our cumulative studies to date indicate that infants and children have CD4 T-cell dysfunction that extends to Th-17 cells (as compared to adults) whether the type of antigenic exposure is by vaccination or naturally (unpublished data).

APC function

The innate immunity is key to protection against infection in early life but are deficient in newborn and preterm infants[78]. Human myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) are the major producers of IL-12p70 and IFN-α/β respectively[79]. IFN-α/β plays a crucial role in anti-viral immunity and assists the Th1 type immune response. IL-12 plays a major function in co-stimulating Th1 immunity[68]. Available evidence suggests that DC–T-cell interactions are the key determinants of induction of adult-like protective Th1 responses in children[17, 33, 79]. Considering the minimal effects of maternal antibodies on T-cell responses, deficiencies in numbers of DCs and their functional incompetence are potentially the crucial factors limiting the capacity to generate T effector-memory responses in early childhood[15, 79, 80]. Despite the suboptimal levels in neonates, DC derived cytokine IL-12 has been shown to modulate antigen specific T-cell responses by inducing T-cell: IFN-γ secretion[17]. IL-12 also mediates the DC directed T-cell differentiation into T follicular helper cells (TFH) which in turn will generate long term B cell memory response[81]. Therefore, suboptimal activation and functional immaturity of DCs in low vaccine responding infants and young children could directly contribute to defective T-cell function and B-cell memory.

Maturational changes in populations of APCs could have an impact on vaccine-specific memory populations and responses to boosters. Dendritic cells (DC), the most potent antigen presenting cells, are the primary activators of naïve T-cells. DCs and naïve T-cell crosstalk provides the opportunity for antigen recognition through T-cell receptor (TCR) interactions with peptide-MHC complexes at the DC surface[82, 83]. TCRs of naïve CD4 T-cells recognize peptides in context to MHC II[84]. After antigen-uptake, DCs mature and up-regulate several accessory molecules, such as MHC II and CD80 and CD86 (costimulatory markers for CD28 on T-cells) that are required to efficiently prime naïve CD4 T-cells[83, 85, 86] and the strength of the TCR signaling regulates Th1/Th2 polarization[84]. The maturation of naïve CD4 T-cell priming into effector/memory cells is also dependent on the cytokine milieu provided by matured DCs and results from toll like receptor (TLR) triggering. TLRs are the main pathogen recognition receptors that are expressed on the cell surface and within intracellular endosomes. Activation of the TLR results in an internal cascade of signaling events that result in gene expression, cytokine/chemokine secretion and cellular activation[87, 88]. Since DCs link the innate immune system to the adaptive immune system by pathogen associated molecular patterns (PAMPs) and T-cell activation (TLRs, cytokines, MHC), adequate priming of naïve CD4 T-cells and generation of effective memory T-cells may be compromised in neonates, infants and young children by inefficient APC function.

TLR mediated activation of DCs leads to maturation and induction of secreted cytokines. Neonatal DCs show an immature phenotype and are functionally impaired in secreting these essential cytokines in response to various TLR agonists[88, 89]. Moreover, TLR expression plays a role in initiating immune activation and differences in TLR expression have been demonstrated to be a component of immunological maturation and adequate immune responses[90]. Immature neonatal innate immunity is associated with poor sepsis outcome [78] but this can be reversed with TLR agonists in murine studies [91] implying that the capacity to respond to TLR stimulation may be a critical factor in low vaccine responding neonates, infants and children. TLR ligation by vaccine antigens, such as the yellow fever vaccine YF-17D, or TLR ligands, promotes cytokine secretion from APCs that in turn leads to T and B-cell activation [92–94]. TLR4 ligation also plays an important role in whole-cell pertussis-mediated protection[95]. However, strong TLR stimulation during early DC differentiation can also lead to tolerogenic APCs[96]. Therefore, it is important to explore TLR expression from a vaccine response perspective (as long-term immunity may be impaired) that could also model neonatal-like APC responses to vaccination.

The failure to outgrow the neonatal immune state among low vaccine responding children may be marked by limited APC function[68]. Low CD28 stimulation by CD80/CD86 on APCs drives neonatal CD4 T-cells into singly functional IL-2^{low} producers[8]. We recently found that APCs of low vaccine responding infants modeled a neonatal state in at least two aspects. Specifically, we found lower expression of MHC II, as shown in Figure 6[26], as well as lower levels of CD80/86 also seen by others [79, 97, 98]. We also found significantly impaired uptake of OVA-FITC in low vaccine responding infants' peripheral blood mDCs and monocytes but not pDCs along with lower APC percentages (data not shown). These data suggest that CD4 T-cell dysfunction in low vaccine responders may be compounded by impaired APCs antigen processing and diminished presentation.

Summary

Our working hypothesis is that LVR children display immune dysfunction and maturational delay similar to neonates and that studying these children and neonates will provide knowledge as to the mechanistic cause of the immune dysfunction in these vulnerable populations eventually resulting in improvements in vaccine strategy. We and others have identified a number of immune targets, such as reduced CD4 T-cell memory and APC function. We see a path forward using TLR agonists or novel adjuvants to stimulate the immune response and overcome poor protective responses in neonates and young children.

Conclusion

Although challenging because of the age of the subjects, more studies to further understand immune dysfunction in neonates, infants and young children are needed. The quality and quantity of systemic and mucosal antibody and memory B-cell generation, adaptive CD4 T-cell vaccine-specific responses and memory recall, and APC – B-cell and CD4 T-cell interactions following recommended vaccinations needs to be more thoroughly characterized. Determining the mechanisms within each cell line responsible for immune maturational delays compared to older children and adults that could be overcome by a more rational approach to modifications in pediatric vaccines such as the addition of novel adjuvants is an achievable goal in the near future.

Finally, a number of groups are using systems biology to identify signatures that can predict immune responses to human vaccines[93, 99, 100] We have initiated experiments to look at gene expression differences among children that suffer from recurrent AOM[101, 102] and are moving to use a systems biology approach to understand the immune defects in children with a neonatal-like immune response to vaccines. The future looks bright that we will able to better understand the immune mechanisms such that newer vaccines will be developed with the goal of being able to offer broader protection against infection to neonates, infants and young children.

Acknowledgments

This work was supported by the U.S. NIH NIDCD RO1 08671. Drs. Robert Zagursky, Naveen Surendran and Saleem Basha, Rochester General Hospital Research Institute, provided useful discussion in preparation of this manuscript.

Abbreviations

DT	diphtheria
TT	tetanus
PT	pertussis toxoid
FHA	pertussis filamentous hemagglutinin
PRN	pertussis pertactin
pDC	plasmacytoid DC

mDC	myeloid DC
sOP	stringent otitis prone
LVR	low vaccine responder
NOP	non-otitis prone
APC	antigen presenting cell
PBMC	peripheral blood mononuclear cells

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Highlights

Neonates and infants produce lower vaccine-specific IgG antibody than older children to vaccines.

T-cell and B-cell memory in neonates and infants is inadequate for prolonged protection.

Antigen presenting cells function inefficiently in processing vaccines in neonates and infatnts.

Low vaccine responders among infants may have a prolonged neonatal-like immune profile.

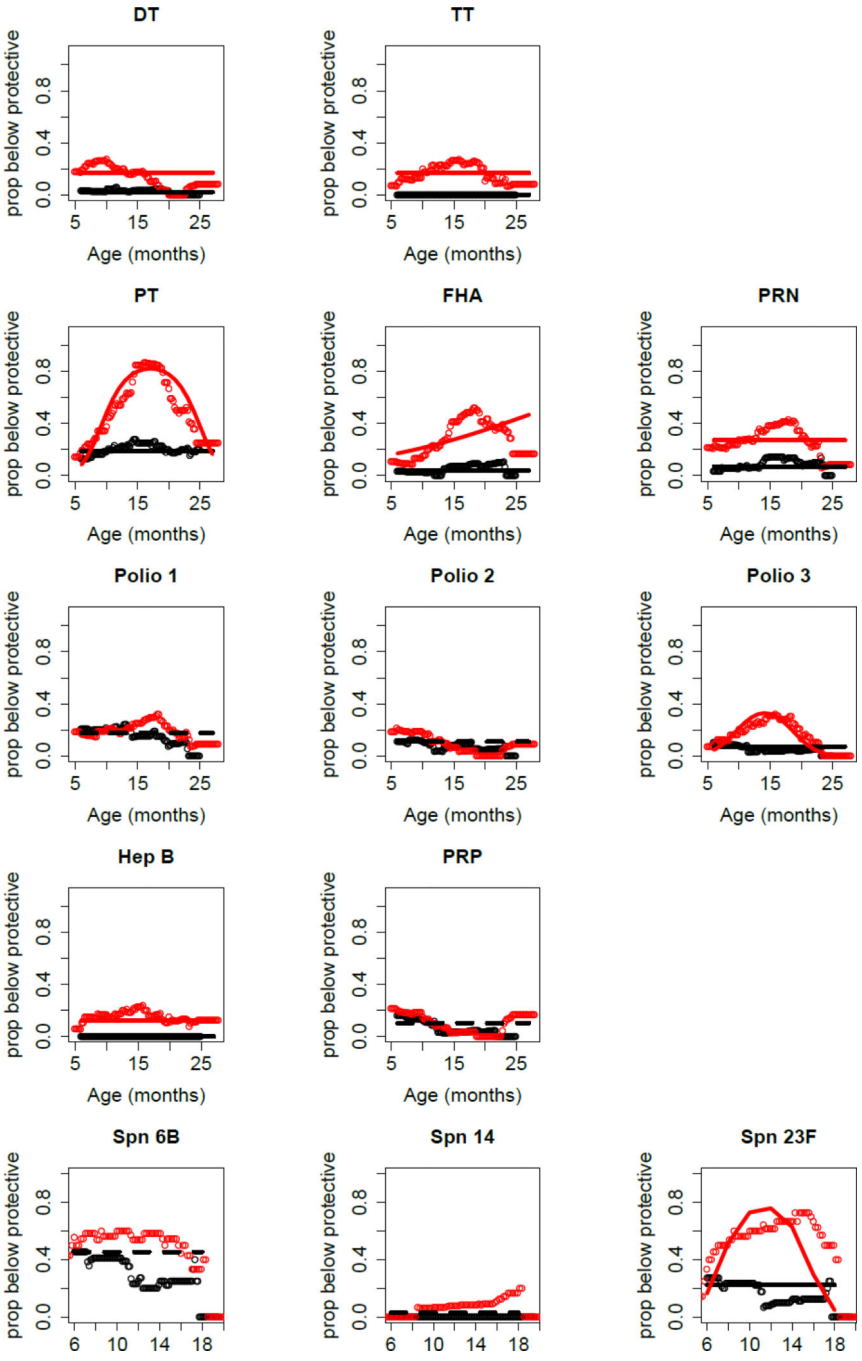

Figure 1. Proportion of age-matched sOP children (n=34; red color) and non-sOP children (n=34; black color) with antibody protective levels plotted against age of the child

sOP children more frequently had nonprotective levels of antibody but no time gradient for DT, TT, PRN and HepB. The sOP children more frequently had nonprotective levels of antibody for PT, FHA, Polio 3 and Spn 23F, but the group effect varied with age. sOP and non-sOP children responded similarly to Polio 1, Polio 2, PRP, Spn 6B and Spn 14. Solid lines: Generalized estimating equations (GEEs) were used to fit the statistical models as previously described[21].

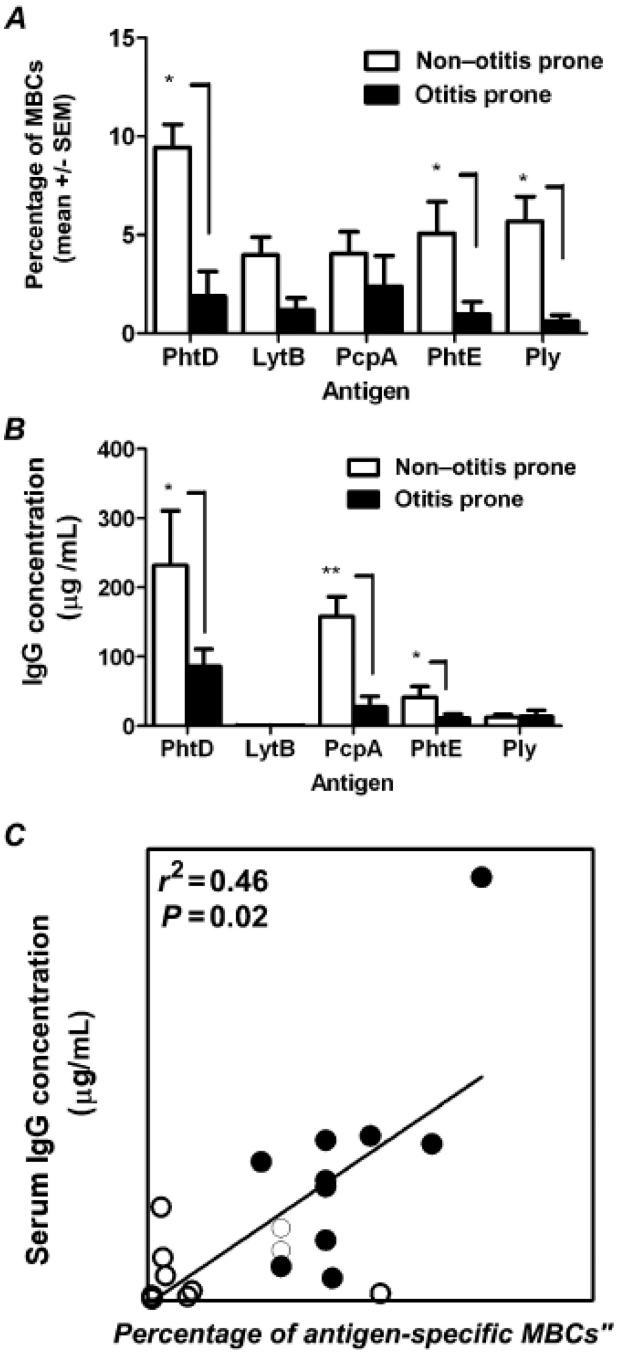

Figure 2. Correlation of antigen-specific memory B cells and serum IgG titers in LVR versus NOP children

(A) Frequencies of antigen-specific memory B cells (MBCs). (B) Serum immunoglobulin G (IgG) titers to 5 pneumococcal protein antigens. (C) Correlation between PhtD-specific serum antibody titers and PhtD-specific percentages of antigen-specific MBCs. Data are for 10 LVRsOP children and 12 non–otitis prone (NOP) children. Open circles denote otitis prone and closed circles denote non–otitis prone. *P = 0.05; ** P = 0.005.

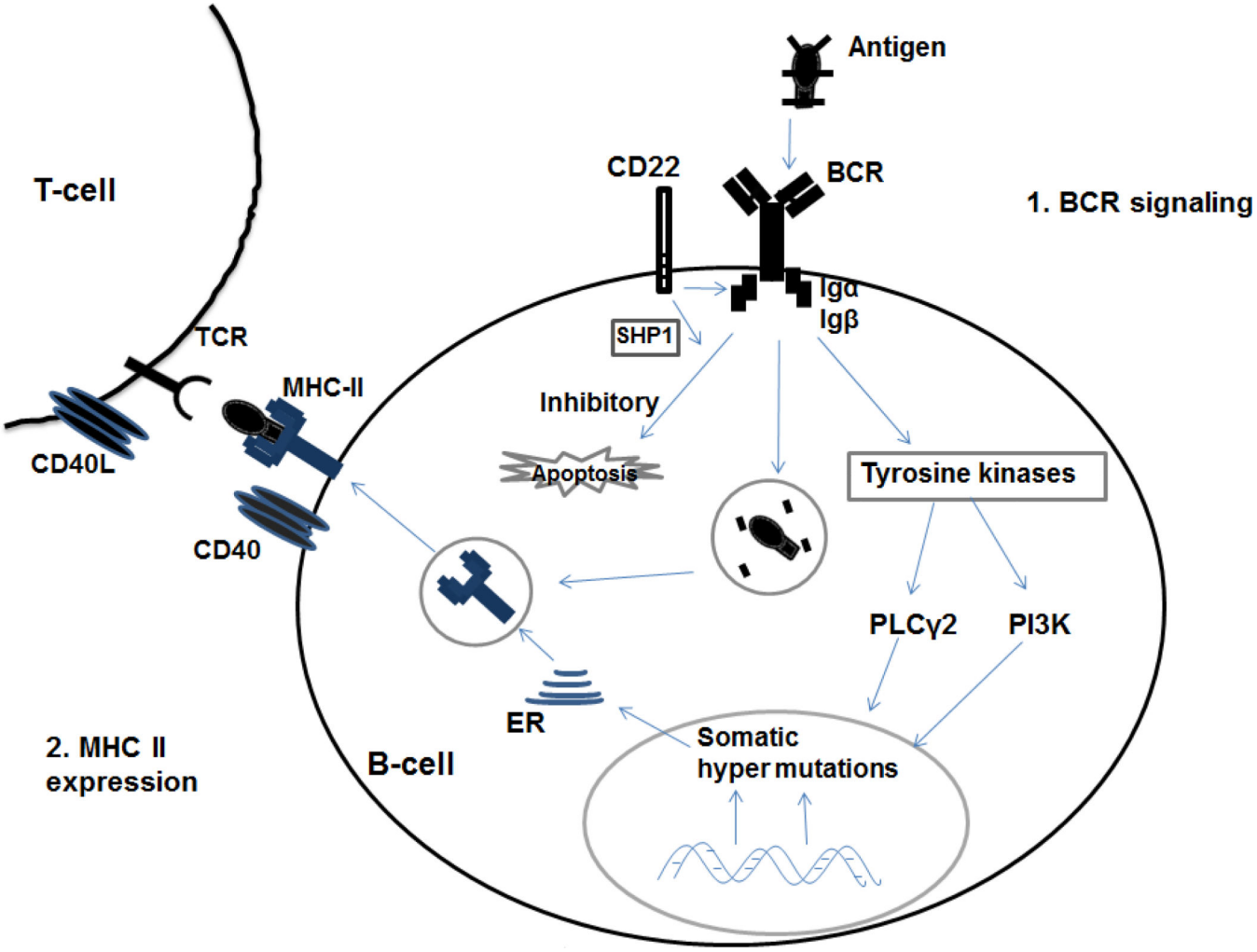

Figure 3. B cell receives proliferation and differentiation signals through the B cell receptor (BCR) and the co-receptors Igα and Igβ

Following antigen binding to BCR, the co-receptors Igα and Igβ signals to activate downstream molecules phospholipase Cγ2 (PLCγ2) and the phosphoinositide 3-kinase (PI3K) pathway. CD22 is a B cell surface glycoprotein that can negatively regulate BCR signaling. Activation of BCR leads to phosphorylation of CD22, resulting in recruitment of SHP-1 to CD22 by Lyn which finally induces the apoptosis pathway. Defects in PLCγ2 or PI3K impair BCR signaling, class-switching responses and memory B cell formation. CD40 is a co-stimulatory marker expressed on B cells and CD40L (CD154) is the ligand expressed on T cells. CD40-CD40L interaction plays a critical role in T cell-dependent B cell antibody response.

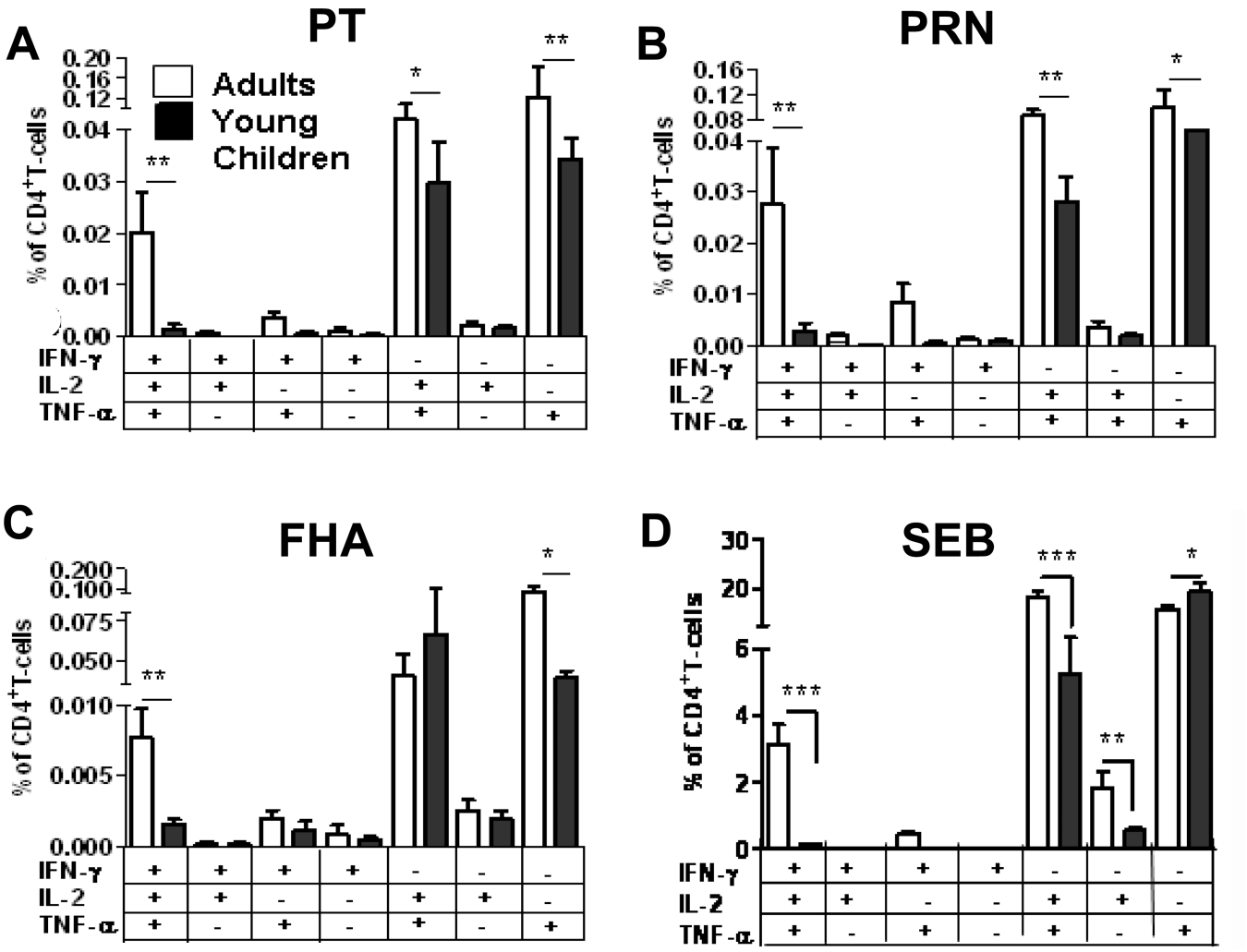

Figure 4. CD4 T-cell responses in young children and adults

We assessed the poly-functional cytokine potential CD4 T-cells (PBMC) responses in young children (<12 months, n=20) and adults (mean age 30yr, n=12) to (A) PT, (B) PRN, (C) FHA, or with (D) SEB stimulation. P values *<0.05 and ***<0.005. (Figure is a regraph of figure 3 in reference[25])

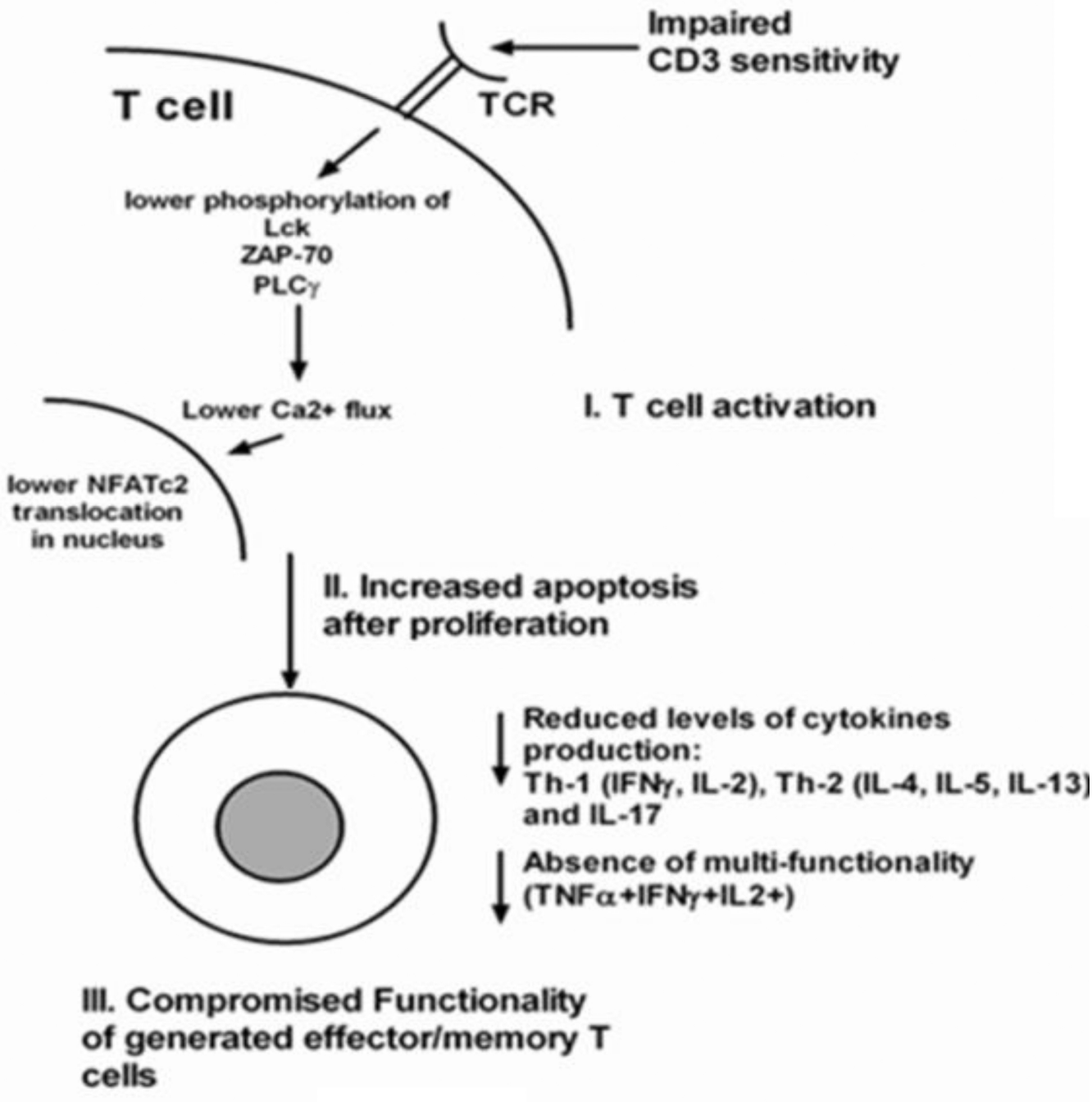

Figure 5. Shortcomings of neonatal and young child T-cells

Various stages in neonatal and young children T-cell activation starting with T-cell receptor (TCR)-mediated activation. T-cells have reduced CD3 sensitivity and lower phosphorylation of several TCR-signaling molecules resulting in low Ca^{2+} flux induction and reduced NFATc2 translocation in the nucleus. After activation, neonates and young children T-cells demonstrate comparable levels of cellular proliferation compared to adult T-cells. However, neonatal and young children T-cells are prone to apoptosis post-activation. The limited

frequencies of activated T-cells that result in effector/memory generation do not produce optimal levels of cytokines and lack multi-functionality.

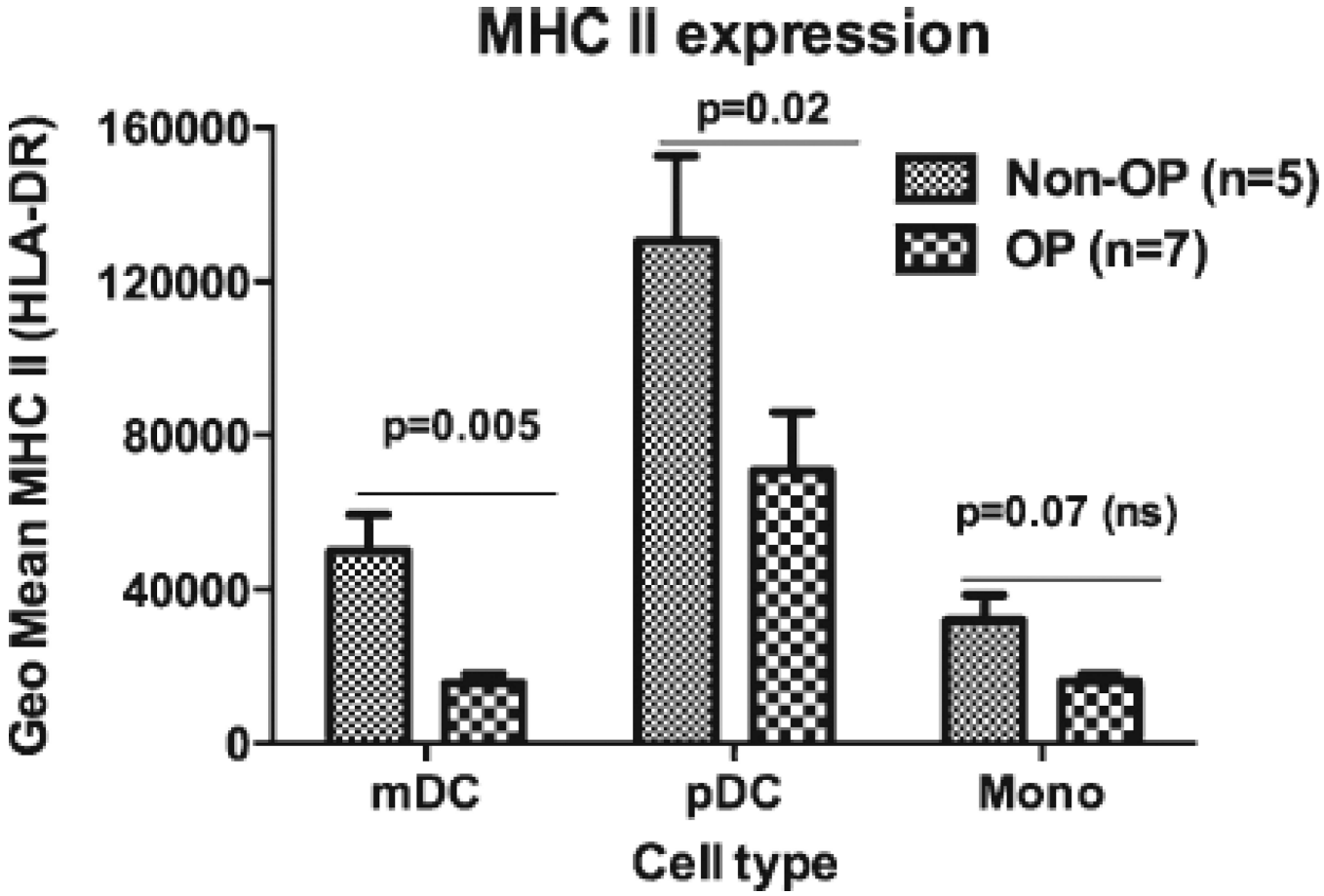

Figure 6. MHC II expression levels on APCs of otitis-prone (OP) and non-otitis-prone (NOP) children

The MHC II levels of the different cell types from peripheral blood were measured using flow cytometry. *mDC*, myeloid dendritic cells; *pDC*, plasmacytoid dendritic cells; *Mono*, monocytes.