Enveloped Virus but not Bacteria Block IL-13 Responses in Human Cord Blood T Cells In Vitro

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Abstract

Infections that occur early in life may have a beneficial effect on the immune system and thereby reduce the risk of allergen sensitization and/or allergic disease. It is not yet clear to what extent specific virus and/or bacteria can mediate this effect. The purpose of this study was to assess the role of virus and bacteria in CD4+ T cell-derived cytokine production in newborns. We compared the effects of five bacteria (Staphylococcus aureus, Escherichia coli, Clostridium difficile, Lactobacillus rhamnosus and Bifidobacterium bifidus) and seven virus (adenovirus, coronavirus, cytomegalovirus, herpes simplex virus, influenza virus, morbillivirus and poliovirus) on the Th1/Th2 cytokine production in mixed lymphocyte reactions using CD4+ T cells from cord blood cocultured with allogenic myeloid or plasmacytoid dendritic cells. When comparing the baseline cytokine production prior to microbial stimulation, we observed that cord plasmacytoid DC were stronger inducers of Th2 cytokines (IL-5 and IL-13) compared with cord myeloid DC and to adult DC. When adding microbes to these cultures, bacteria and virus differed in two major respects; Firstly, all enveloped viruses, but none of the bacteria, blocked Th2 (IL-13) production by cord CD4+ cells. Secondly, all Gram-positive bacteria, but none of the virus, induced IL-12p40 responses, but the IL-12p40 did not affect Th1 cytokine production (IFN-γ). Instead, Th1 responses were correlated with the capacity to induce IFN-γ secretion, which in cord cells were induced by S. aureus and influenza virus alone. These data imply that enveloped virus can deviate Th2 responses in human cord T cells.

Introduction

Allergic diseases among children and youth are one of the most common chronic diseases in the Western world and the prevalence has increased drastically during the last 40 years [1]. The hygiene hypothesis states that a reduced exposure to microbes increases the risk of developing allergies. This hypothesis was originally based on observations showing that children with many siblings, children attending early day care or children growing up in poverty are less prone to develop allergies [2]. It is, however, not yet clear which microbes that can and cannot affect allergy development.

Epidemiological studies show that certain viral and bacterial infections correlate with a reduced incidence of allergic manifestations. We have recently shown that infection with human herpes virus type 6 (HHV-6) is associated with reduced allergic sensitization in 18-month-old children [3]. We have confirmed this in an experimental animal model of allergic asthma, where mice that are exposed to HHV-6 are protected against allergic inflammation. Mice exposed to HHV-6 have significantly lower levels of allergen-specific IgE, eosinophils and Th2 cytokines as compared to allergic control mice [4]. In addition, previous infection with EBV [5, 6] and Hepatitis A virus [7, 8] has been associated with a reduced incidence of allergic sensitization and allergic symptoms in human subjects. Infection with orofecal and foodborne bacteria, including Toxoplasma gondii and Helicobacter pylori, or exposure to bacterial components, such as endotoxin, have also been demonstrated to be inversely related to atopic allergy [8–11]. Furthermore, the composition of the intestinal commensal flora has been suggested to affect the risk of developing allergic disease, where early colonization with bifidobacteria and lactobacilli is associated with a lower prevalence of allergy in young children (0–2 years of age) [12–14].

The allergic response is driven by Th2 cells, and their secretion of IL-4, IL-5 and IL-13. The initiation of the T cell response and the subsequent maturation of the
T cells, including their differentiation into Th1 or Th2 cells, are regulated by dendritic cells (DC) [15]. These cells are generally divided into two major subsets; myeloid CD11c⁺CD123⁻ DC (mDC) and plasmacytoid CD11c⁺CD123⁺ DC (pDC). MDC are the main source of IL-12, which is pivotal in the differentiation of naïve CD4⁺ T cells into the favoured Th1 phenotype [16–18].

PDC tend to be more prone to induce Th2 responses compared with mDC [15], by inducing IL-4, IL-5 and IL-13 in responding T cells [19]. However, the Th2-skewing effect of pDC can be omitted by viral exposure or binding of CpG to TLR-9 [3, 19].

In contrast to the adult immune system, the immune system of newborns is immature, which include impairments in both innate and acquired immune responses. This is largely due to a poor DC function in the newborns [20], which is accompanied with a reduced capacity to produce the Th1-polarizing cytokines IL-12 [21, 22], IFN-γ [21, 23] and IFN-β [24]. Even though pDC from cord blood have impaired IFN-γ/β production after TLR activation [23], cord pDC may secrete large amounts of IFN-α after viral exposure. We have recently shown that cord pDC exposed to HHV-6 produce large amounts of IFN-α. This was correlated with a reduced capacity to induce IL-5 and IL-13 in responding T cells, which instead produced elevated levels of IFN-γ [3]. Thus, repeated microbial stimuli of the innate immune system of neonates may accelerate the maturation process and enhance Th1 cell development. The amplified Th1 responses might then lead to reduced Th2 polarization and a reduced risk of developing allergic diseases, in line with the hygiene hypothesis [25]. In addition, the immune system of newborns is also characterized by less mature regulatory T cells [26] that have a reduced suppressive capacity [27]. Still, regulatory T cells of the neonatal immune system are functional and able to exert suppressive functions [28, 29], yet to a lesser extent than those in adults [27].

The purpose of this study was to evaluate how different microbes affect T cell activation in cord cells. For this purpose, five different bacteria and seven different viruses were used. Bacteria were chosen based on (i) being Gram-negative or Gram-positive bacteria and (ii) being part of the commensal intestinal flora and/or the cause of infection in humans [30]. The viruses were chosen based on (i) being dsDNA, tRNA or ssRNA viruses, (ii) being enveloped or non-enveloped and (iii) causing either acute or chronic infection in humans. To study the effect of these microbes, we measured cytokine secretion in cord blood-derived T cells that were cultured with all-ogenic pDC or mDC. We found that all enveloped virus tested, but none of the bacteria, could block IL-13 production in cord blood CD4⁺ T cells. This effect was not associated with enhanced Th1 responses. Our data suggest an important role for enveloped viruses in the early maturation of the immune system.

**Material and methods**

**Virus.** Herpes simplex virus type 1 (HSV-1), coronavirus, cytomegalovirus (CMV) are enveloped, GAG-binding, DNA viruses. Morbillivirus and Influenza A virus are enveloped, sialic acid-binding, RNA virus. Poliovirus is a naked RNA virus, and adenovirus is a naked DNA virus.

All viruses were quantified using Real-time PCR (RT-PCR) (TaqMan; Applied Biosystems, Foster City, CA, USA). HSV-1, CMV and adenovirus were quantified using quantification standards that were generated using a plasmid containing a cloned insert of the target sequence. Primers and probes were used as previously described [31–33]. The methods, primers and probes used for the quantification of coronavirus [34], poliovirus [35] and influenza A [36] were used as previously described. Morbillivirus was quantified using forward primer 5’-CGT TGA CCC TGA CGT TAG CA -3’, reverse primer 5’-GCC GAA GTC AAG CCA GAT TG-3’ and the probe sequence was 5’-GTC CTC AGT AGT ATG CAT TGC AA-3. All viruses were inactivated at 2500 rad and stored at −70 °C before use.

**Bacterial strains.** The bacterial strains were isolated from stool samples of Swedish infants obtained at 3 days–8 weeks of age. Staphylococci were isolated on staphylococcus agar and identified as *Staphylococcus aureus* using the coagulate test. A *S. aureus* isolate that produced enterotoxin A and toxic shock syndrome toxin-1 (TSST-1), but not enterotoxins B, C or D, was tested for enterotoxin production using the SET-RPLA kit, and for TSST-1 using the TST-RPLA kit (both kits from Oxoid, Hampshire, UK). *Escherichia coli* was isolated on Drigalski agar (Media Department, Gothenburg University, Sweden) and was identified using the API20E biotyping system (bioMérieux Industry, Marcy l’Etoile, France). *B. bifidus* was isolated on Beerens agar (Media Department) and identified by genus-specific PCR. *Lactobacillus rhamnosus* was isolated on Rogosa agar (BD Diagnostics), and *Clostridium difficile* was isolated from alcohol-treated samples and identified using the RAPID ID 32A system (bioMérieux Industry). Prior to use in cell culture, all strains were counted in a microscope and inactivated by exposure to UV-light for 20–30 min. Inactivation was confirmed by negative viable counts and the bacteria were stored at −70 °C until use.

**Purification of cells.** Cord blood was obtained from unselected healthy infants. Buffy coats were obtained from the blood central at Sahlgrenska University Hospital. Cells were isolated by density gradient centrifugation over Ficoll–Paque (GE Healthcare Bio-sciences AB, Uppsala, Sweden). Fresh pDC and mDC were isolated from cord and adult blood using the pDC isolation kit CD304 (BDCA-4) (purity: 79–92%) and the mDC isolation kit CD1c (BDCA-1) (purity: 85–96%), both from Miltenyi.
Biotec (Auburn, CA, USA). The mean yield for pDC and mDC were 0.34% (range: 0.14–0.6%) and 1.1% (range: 0.42–1.45%), respectively. CD4+ T cells were isolated from cord and adult blood using the Dynal CD4+ isolation kit (Invitrogen Dynal AS, Oslo, Norway) (purity: >95%). All separations were carried out according to the manufacturer’s instructions.

Mixed lymphocyte reaction. To study the impact of APC on T cell responses, 10⁵ adult CD4+ T cells were cultured with 2 x 10⁴ allogenic adult pDC or adult mDC (n = 10 donors) in 96-well flat-bottomed Nunc tissue culture plates (Thermo Fisher Scientific, Roskilde, Denmark) in Iscoves complete medium (supplemented with 10% FBS, 1% l-glutamine, 1% gentamicin and 1% 2-ME). 10⁵ cord CD4+ T cells were cultured with 2 x 10⁴ allogenic cord pDC or cord mDC (n = 13 donors) in 96-well flat-bottomed Nunc tissue culture plates in Iscoves complete medium. The different viruses were added at a concentration of 70 genome copies/DC. The different bacteria were added at a concentration of 100 bacteria/DC. Supernatants were collected after 48 h and frozen in −20°C until use. Figure 1 depicts a schematic overview of the study design.

Cytokine determination. ELISA. Flat-bottomed Maxisorp 96F microwell plates (Nunc A/S, Roskilde, Denmark) were coated overnight at 4°C with an anti-human IL-12 p40 antibody, diluted according to instructions. The plate was read at 450 nm using SPECTRAMAX 340 PC and SOFTMAX PRO 5.2. Concentrations lower than 10 pg/ml was considered as negative.

IL-13 levels were determined using an in-house IL-13 ELISA. Flat-bottomed Maxisorp 96F microwell plates (Nunc A/S) were coated overnight at 4°C with an anti-human IL-13 monoclonal antibody in a concentration of 2 μg/ml (BD Biosciences, Pharmingen, San Jose, CA, USA), which was followed by 1 h of blocking with 0.5% BSA in room temperature. Samples and human IL-13 standards were added and incubated for 1 h at room temperature, and the plates were then consecutively incubated for 1 h at room temperature with a biotinylated detection antibody in a concentration of 1 μg/ml (BD Biosciences, Pharmingen) followed by streptavidin poly HRP (Sanquin, Amsterdam, Netherlands). Plates were then developed using 0.1 mg/ml TMB (Sigma–Aldrich) in 0.05% phosphate–citrate buffer, pH 5.0 and 0.04% H₂O₂ followed by 1 M H₂SO₄. Absorbance was measured at 450 nm using SPECTRAMAX 340 PC and SOFTMAX PRO 5.2. Concentrations lower than 10 pg/ml was considered as negative.

IFN-γ levels was determined using an in-house IFN-γ ELISA kit from PBL InterferonSource (Piscataway, NJ, USA) that detect 14 of 15 isoforms of hIFN-γ. These include IFN-γA, IFN-γB2, IFN-γC, IFN-γD, IFN-γE, IFN-γF, IFN-γG, IFN-γH, IFN-γJ, IFN-γK, IFN-γL, IFN-γM, and IFN-γN. Concentrations lower than 10 pg/ml was considered as negative.

Cytometric bead array. IFN-γ, IL-2 and IL-5 content were determined using the Human Th1/Th2 Cytokine Cytometric Bead Array kit according to manufacturer’s instructions (BD Biosciences, Pharmingen). Briefly, 20 μl of capture beads were added to a V-bottomed 96-well plate together with 20 μl of the unknown samples or the Th1/Th2 standard in two-fold serial dilutions (top concentration: 5000 pg/ml) and 20 μl of the human Th1/Th2 –II PE detection antibody. The plate was then incubated for 3 h in the dark at room temperature, where after 200 μl of washing buffer was added and the plate was centrifuged at 200 g for 5 min. The supernatants were removed and the pelleted beads were resuspended in 300 μl of washing buffer and analysed on a FacsCanto2 flow cytometer. The data were analysed using the FCAP array software (BD Biosciences, Pharmingen). All given

![Figure 1](image-url)
values calculated from the standard curve were considered as positive. For all cytokine measurements, undetected samples were set as 1 pg/ml.

Statistical analysis. Statistical analyses were performed using one-way ANOVA followed by Bonferroni or Dunnett’s multiple comparison tests for GRAPHPAD PRISM (La Jolla, CA, USA).

Ethics. This study was approved by the Ethics Committee in Gothenburg, Sweden.

Results

Cord pDC promote Th2 cytokine production

The first question we addressed was whether CD4+ T cells respond differently to adult and cord mDC and pDC. As cord T cells have not yet met a specific antigen, it is not possible to measure recall T cell responses in these cells. Instead, we assessed the cytokine profiles in cord T cells in response to allogenic DC, that is in a mixed lymphocyte reaction (MLR). We, therefore, incubated purified cord blood CD4+ T cells with allogenic cord mDC or cord pDC and analysed the cytokine profile after 48 h of coculture. Similarly, adult CD4+ T cells were incubated with allogenic adult mDC or adult pDC, and the cytokine profile was assessed after 48 h of coculture. The cytokines analysed were the Th1-specific cytokines IL-2 and IFN-γ and the Th2 cytokines IL-5 and IL-13. We found that pDC from cord blood induced significantly higher levels of the Th2 cytokines IL-5 and IL-13 in responding CD4+ T cells compared with both pDC and mDC from adult blood and to mDC from cord blood (Fig. 2C,D). Cord pDC induced 8.5-fold higher levels of IL-13 and 19-fold higher levels of IL-5 compared with adult pDC, and five-fold and 13-fold higher levels of these cytokines compared with cord mDC. We could not detect any differences in Th2 cytokine production when comparing mDC from cord and adult blood (Fig. 2C,D). Furthermore, cord pDC also induced higher levels of the Th1 cytokines IL-2 and IFN-γ compared with adult pDC and compared to mDC from both adult and cord blood (Fig. 2A,B). These differences were however not statistically significant.

Enveloped viruses block IL-13 secretion in cord CD4+ T cells

To assess the role of bacterial and viral stimuli in Th2 differentiation, CD4+ T cells from cord blood were assayed in an MLR together with different strains of bacteria and virus. To compare the effect of the different microbes on cytokine secretion, we assessed the relative change in cytokine production for each microbe. The relative change was calculated using the amount of cytokine produced in MLR cultures containing a specific microbe, divided by the cytokine amount secreted in an MLR lacking microbe. All enveloped viruses tested (coronavirus, CMV, HSV-1, influenza virus and morbillivirus) downregulated the IL-13 responses in cord blood cocultures (Fig. 3E,F). The non-enveloped viruses, adenovirus and poliovirus had no effect on the IL-13 production in cord MLR cultures using either pDC (Fig. 3F) or mDC (Fig. 3E) from cord blood as antigen presenting cells. Neither did any of the bacteria reduce the IL-13 responses. Instead, S. aureus stimulated pDC increased the

Figure 2 Increased baseline levels of Th2 cytokine production in cord pDC stimulated CD4+ T cells. CD4+ T cells from cord blood or adult peripheral blood were cultured with allogenic pDC or mDC. Supernatants were collected after 48 h and analysed for IL-2 (A), IFN-γ (B), IL-5 (C) and IL-13 (D). Data represent the individual and the mean of secreted cytokines in picogram per millilitre from 10 (adult) to 13 (cord) MLRs using One-way ANOVA followed by Bonferroni multiple comparison test.
IL-13 production in responding CD4+ T cells (Fig. 4F). We were not able to document any significant inhibitory effects on the IL-5 production by the virus, most likely due to the very low initial production of this cytokine (not shown).

Influenza A and S. aureus affect Th1 cytokine secretion

The effect of viral and bacterial stimuli on Th1 cytokine secretion was assessed using cord CD4+ T cells cocultured with allogenic pDC or mDC from cord blood. Both bacteria and virus could affect IL-2 and IFN-γ secretion by cord CD4+ T cells (Figs 3 and 4). Influenza virus was the most efficient inducer of IL-2 and significantly enhanced the responses in cord CD4+ T cells exposed to cord pDC (Fig. 3B) and to cord mDC (Fig. 3A). Influenza virus also enhanced the IFN-γ responses, but only in cord T cell/mDC cultures (Fig. 3C). None of the other viruses tested affected the IL-2 or IFN-γ production in these cocultures except CMV that reduced the IL-2 production from cord CD4+ T cells and pDC cocultures (Fig. 3B), that is from the cells with the highest initial IL-2 production (Fig. 2A). Staphylococcus aureus was the only bacteria that enhanced IL-2 responses by cord CD4+ T cells exposed to both mDC (Fig. 4A) and pDC (Fig. 4B). Staphylococcus aureus was also a potent inducer of IFN-γ responses in both pDC and mDC stimulated cord CD4+ T cells (Fig. 4C,D).
Microbe-induced Th1 responses correlate with IFN-α but not IL-12 p40 responses

To assess innate cytokine secretion in cord DC, pDC and mDC from cord blood were stimulated with different strains of bacteria and virus together with allogenic cord CD4+ T cells. We found that all Gram-positive bacteria, but not E. coli or any of the viruses, promoted an IL-12 p40 response in MLR cultures with mDC (Fig. 5A,C) but not with pDC (not shown). The increase in IL-12 production in C. difficile stimulated cell cultures was, however, not statistically significant, even though there was a strong trend (Fig. 5A). We also analysed the ability of virus and bacteria in evoking an IFN-α response in pDC. The only microbes that were able to induce INF-α secretion were S. aureus (Fig. 5B) and influenza virus (Fig. 5D), that is the only two microbes that promoted IL-2 and IFN-γ responses.

Discussion

In this study, we show that cord pDC promote a Th2 phenotype. However, the Th2-skewing effect of cord pDC could be omitted by enveloped viruses. This implies that virus can divert Th2-biased responses in human cord T cells. Furthermore, we show that microbes capable of inducing IFN-α promote Th1 responses, whereas a microbe’s ability to induce IL-12 does not correlate to its ability to induce IL-2 or IFN-γ responses in vitro.

The numbers of human studies of adaptive T cell responses in newborns compared with adults are limited...
and conflicting [37]. Yet, it is generally thought that the immune system of newborns is immature and differs from that in adults. The T cell polarization in newborns is correlated with impaired Th1 responses [38, 39]. However, individual Th1/Th2 balance in newborns varies depending on parental and environmental factors [40]. In this paper, we show that the baseline production of the Th2 cytokines IL-5 and IL-13 were elevated in cord CD4+ T cells compared with adult T cells. The Th2 cytokine induction observed in cord cells was not an intrinsic function of the neonatal T cells, but rather a Th2-inducing effect of cord pDC. This is in line with previous findings where pDC was shown to promote Th2 responses in healthy and allergic subjects [15, 19]. This is, to our knowledge, the first study to show that the levels of Th2 cytokines obtained in vitro activated T cells differs between newborns and adults. We could not detect any significant differences in Th1 cytokine synthesis (IFN-γ and IL-2) between T cells from adults and newborns, even though others have shown that cord blood DC is impaired in their capacity to induce both IFN-γ and IL-2 in responding T cells [39]. Instead, our data imply that cord pDC were superior to both cord mDC and adult DC in promoting Th2 responses.

The Th2-skewing effect of cord pDC can be blocked by viral stimuli. We found that enveloped viruses (i.e. HSV-1, coronavirus, CMV, morbillivirus and influenza virus) blocked IL-12 secretion, while bacteria and non-enveloped viruses did not. This confirms previous findings from us and others, showing that the Th2 skewing effect of pDC in newborns and adults can be omitted by microbial stimuli [3, 19]. However, the diminished IL-13 production that was seen in virus stimulated cultures could not be correlated with Th1 polarization, that is IFN-α, IFN-γ, IL-2 or IL-12 secretion. None of the viruses tested could induce IL-12 secretion, and influenza was the only inactivated virus to evoke IFN-α, IFN-γ and IL-2 production. Still, these findings emphasize the importance of early life microbial stimuli of the innate immune system for an accurate maturation of the immune system, that is to avoid unwanted Th2 responses. We propose that a crucial component of this maturation process is an appropriate activation of cord pDC.

Gram-positive bacteria were the only of the microbes tested that induced IL-12 secretion, and only in mDC cultures, which is consistent with previous findings in both cord and adult cells [41, 42]. However, IL-12 secretion could not be correlated with the induction of Th1 cytokine secretion, as S. aureus was the only microbe to induce both IL-12 and Th1 cytokine secretion. As we only measured IL-12 p40 and not the biologically active IL-12 p70, we cannot deduce from this study whether any of the tested bacteria did indeed induce IL-12 p70. However, Gram-positive bacteria are known for their capacity to induce IL-12 p70 in both adults and newborns [41, 42]. Yet, others have shown that the synthesis of IL-12 p70 is impaired in newborns [21, 43] and that lymphocytes from cord blood lack IL-12 receptor β1 expression [44], which may explain the absent correlation between IL-12 secretion and Th1 cytokine secretion. Furthermore, the use of UV-inactivated bacteria could also explain the lack of IL-12 secretion in bacteria stimulated cultures. However, it has previously been shown that live
with HHV-6 or EBV is inversely related to allergic sen-
sis in responding cord T cells

skewed effect. This is in line with our previous findings

that cord pDC drive the neonatal T cell response towards

Th2 bias and that viral stimuli can omit this Th2

response. In this paper, we show

driving the adaptive immunity away

from Th2 biased immune responses, and thus, to prohibit

the development of allergic diseases.

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References

1 Bach JF. The effect of infections on susceptibility to autoimmune

and allergic diseases. N Engl J Med 2002;347:911–20.

2 Strachan DP. Hay fever, hygiene, and household size. BMJ 1989;299:1259–60.

3 Nordstrom I, Rudin A, Adlerberth I et al. Infection of infants with human herpesvirus type 6 may be associated with reduced allergic

sensitization and T-helper type 2 development. Clin Exp Allergy 2010;40:882–90.

4 Svensson A, Almqvist N, George Chandy A, Nordstrom I, Eriksson K. Exposure to Human Herpes virus type 6 protects against allergic

asthma in mice. J Allergy Ther 1 2010;101:1–7.

5 Nilsson C, Larson Sigfrinius AK, Montgomery SM et al. Epstein-

Barr virus and cytomegalovirus are differentially associated with numbers of cytokine-producing cells and early atopy. Clin Exp

Allergy 2009;39:509–17.

6 Nilsson C, Linde A, Montgomery SM et al. Does early EBV infection protect against IgE sensitization? J Allergy Clin Immunol 2005;116:438–44.

7 Matricardi PM, Rosmini F, Frittigno L et al. Cross sectional retrospective study of prevalence of atopy among Italian military students

with antibodies against hepatitis A virus. BMJ 1997;314:999–1003.

8 Matricardi PM, Rosmini F, Panetta V, Frittigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States. J Allergy Clin Immunol 2002;110:381–7.

9 Matricardi PM, Rosmini F, Riondino S et al. Exposure to food-

borne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ 2000;320:412–7.

10 Braun-Fahrländer C, Riedler J, Herz U et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869–77.

11 Ege MJ, Mayer M, Normand AC et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701–9.

12 Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp

Allergy 1999;29:911–26.

13 Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy develop-

ment and the intestinal microbiota during the first year of life. J Allergy Clin Immunol 2001;108:516–20.

14 Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskimies P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 2001;357:1076–9.

15 Risoa MC, Soumelis V, Kadowaki N et al. Reciprocal control of T helper cell and dendritic cell differentiation. Science 1999;283:1183–6.

16 Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O’Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 1999;280:547–9.

S. aureus and E. coli are equally effective in inducing

IL-12 as dead bacteria of the same species, at least in

monocytes from adult blood [42].

Indeed, we found that Th1 cytokine induction was

correlated with IFN-α secretion, which is in line with

previous findings in adults [19, 45–47]. The only two

microbes, influenza virus and S. aureus, that induced Th1

cytokine secretion in cord pDC were also potent inducers

of IFN-α. Our previous findings [3], and this paper,

thus show that pDC from newborns can secrete large

amounts of IFN-α upon stimulation with certain selected

microbes.

The use of non-replicating virus instead of replication-

competent virus may of course explain why some of

the virus tested did not induce any IFN-α/β responses. Yet,

HSV-1 did not induce any IFN-α in cord pDC despite

the ability of replication-deficient HSV in inducing

strong type I interferon responses in adult cells [48, 49].

However, cord pDC have an impaired IFN-α/β signalling

capacity [23], which is as a result of a defect in interferon

regulatory factor (IRF)-7-mediated responses in pDC from

newborns [50]. This could explain why HSV-1, which

bind and signal via TLR-9, was refractory in activating

cord pDC and perhaps also explain why some of the other

viruses tested did not promote IFN-α responses.

There is increasing evidence that the cytokine pattern

in newborns is associated with the propensity to develop

allergic disease. Studies suggest that children that
develop allergies later in life and/or with a family history

of allergy are Th2 skewed at birth, even though conflicting

data exists [38, 51–54]. Elevated levels of IL-13

[55–57] and decreased levels of IFN-γ [51, 58, 59] in

cord T cells has been shown to be risk factors for develop-
ing allergic disease later in life, even though the role

of IFN-γ is less clear-cut [55]. In this paper, we show

that cord pDC drive the neonatal T cell response towards

a Th2 bias and that viral stimuli can omit this Th2

skewed effect. This is in line with our previous findings

where HHV-6 activated pDC block Th2 cytokine synthe-

sis in responding cord T cells [3]. This fits well with our

and others observations, showing that childhood infection

with HHV-6 or EBV is inversely related to allergic

sensitization and/or allergic symptoms [3, 5, 6]. Further-

more, the hygiene hypothesis postulates that the increase

in allergic diseases during the last decades is caused by a

decreased infectious burden [2], which in turn is owing
to vaccination, antibiotics, improved hygiene and gen-

erally enhanced socioeconomic standard [1]. Given that

many childhood viral diseases have a reduced incidence

[1, 60–62], it is tempting to speculate that the large

increase in allergic diseases could be related to a
decreased exposure to viral infections. Taken into account

that our studies were performed in vitro using inactivated

microbes, we suggest that viral infections during infancy

may play an important role in the development of the

immune system, by driving the adaptive immunity away

from Th2 biased immune responses, and thus, to prohibit

the development of allergic diseases.
Macatonia SE, Hosken NA, Litton M et al. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4 + T cells. J Immunol 1995;154:5071–9.

Manetto R, Parrocchi P, Giudizi MG et al. Natural killer cell stimulatory factor (interleukin 12 (IL-12)) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th2 cells. J Exp Med 1993;177:1199–204.

Farkas L, Kvale EO, Johansen FE, Jahnssen FL, Lund-Johansen F. Plasmacytoid dendritic cells activate allergen-specific TH2 memory cells: modulation by CpG oligodeoxynucleotides. J Allergy Clin Immunol 2004;114:446–53.

Willems F, Vollstedt S, Suter M. Phenotype and function of neonatal DC. Eur J Immunol 2009;39:26–35.

Belderbos ME, van Bleek GM, Levy O et al. Skewed pattern of Toll-like receptor 4-mediated cytokine production in human neonatal blood: low LPS-induced IL-12p70 and high IL-10 persist throughout the first month of life. Clin Immunol 2009;135:228–37.

Nguyen M, Leuridan E, Zhang T et al. Acquisition of adult-like TLR4 and TLR9 responses during the first year of life. PLoS ONE 2010;5:e10407.

De Witz D, Olislagers V, Gorisly S et al. Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. Blood 2004;103:1030–2.

McKenzie SE, Kline J, Douglas SD, Polin RA. Enhancement of the innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol 2007;7:379–90.

Wing K, Ekmark A, Karlsson H, Rudin A. Soluble CD14 and CD83 from human neonatal antigen-presenting cells are inducible by commensal bacteria and suppress allergen-induced human neonatal Th2 differentiation. Infect Immun 2007;75:4097–104.

Kullberg-Linth C, Olofsson S, Brune M, Lindh M. Comparison of serum and whole blood levels of cytomegalovirus and Epstein-Barr virus DNA. Transpl Infect Dis 2008;10:308–15.

Namvar L, Olofsson S, Bergstrom T, Lindh M. Detection and typing of herpes simplex virus (HSV) in mucocutaneous samples by TaqMan PCR targeting a gB segment homologous for HSV types 1 and 2. J Clin Microbiol 2005;43:2058–64.

Heim A, Elmer C, Harste G, Fringer-Akerblom P. Rapid and quantitative detection of human adenosine DNA by real-time PCR. J Med Virol 2003;70:228–39.

Gunson RN, Collins TC, Carman WF. Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. J Clin Virol 2005;33:341–4.

Brittain-Long R, Nord S, Olofsson S, Westin J, Anderson LM, Lindh M. Multiplex real-time PCR for detection of respiratory tract infections. J Clin Virol 2008;41:53–6.

Ward CL, Dempsey MH, Ring CJ et al. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. J Clin Virol 2004;29:179–88.
subsequent development of atopic disease in infants. *Pediatr Res* 2002;51:195–200.

56 Lange J, Ngoumou G, Berkenheide S *et al.* High interleukin-13 production by phytohaemagglutinin- and Der p 1-stimulated cord blood mononuclear cells is associated with the subsequent development of atopic dermatitis at the age of 3 years. *Clin Exp Allergy* 2003;33:1537–43.

57 Spinozzi F, Agea E, Russano A *et al.* CD4⁺ IL13⁺ T lymphocytes at birth and the development of wheezing and/or asthma during the 1st year of life. *Int Arch Allergy Immunol* 2001;124:497–501.

58 Kondo N, Kobayashi Y, Shinoda S *et al.* Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders – 6-year follow-up study. *Clin Exp Allergy* 1998;28:1340–4.

59 Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994;344:983–5.

60 Hinman AR, Orenstein WA, Schuchat A. Vaccine-preventable diseases, immunizations, and MMWR – 1961–2011. *MMWR Surveill Summ* 2011;60 (Suppl. 4):49–57.

61 Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine* 2010;28:6653–7.

62 Joussemet M, Depaquit J, Nicand E *et al.* [Fall in the seroprevalence of hepatitis A in French youth]. *Gastroenterol Clin Biol* 1999;23:447–52.