Development of Interleukin-12-Producing Capacity throughout Childhood

John W. Upham,[1,2]* Peter T. Lee,[1]† Barbara J. Holt,[1] Tricia Heaton,[1] Susan L. Prescott,[1,3]
Mary J. Sharp,[1] Peter D. Sly,[1] and Patrick G. Holt[1]

*Telethon Institute for Child Health Research and Centre for Child Health Research[1] and Departments of
Medicine[2] and Paediatrics,[3] University of Western Australia, Perth, Australia*

Increasing evidence indicates that the capacity to induce protective Th1 immune responses is impaired in early childhood, an observation that can be partially attributed to deficiencies in antigen-presenting-cell function. Synthesis of interleukin 12 (IL-12), a key Th1-trophic cytokine, is markedly reduced in the neonatal period, though there is a paucity of knowledge concerning the ontogeny of IL-12-synthetic capacity throughout the childhood years. Hence, we examined the production of bioactive IL-12 p70 by circulating mononuclear cells in a population of healthy individuals. As expected, the capacity to synthesize IL-12 p70 in response to either lipopolysaccharide or heat-killed *Staphylococcus aureus* was markedly impaired at birth, even after priming of cells with gamma interferon. Surprisingly however, IL-12 p70 synthesis by peripheral blood mononuclear cells from both 5- and 12-year-old children was still substantially below that seen in adults, and this did not appear to be related to excessive production of IL-10. In contrast, dendritic cells from adults and neonates, derived from monocytes with granulocyte-macrophage colony-stimulating factor and IL-4, synthesized equivalent amounts of IL-12 p70 in response to microbial stimulation. This indicates that the impaired capacity for IL-12 synthesis in childhood is not an intrinsic property of circulating mononuclear cells but rather can be readily overcome in response to appropriate maturational stimuli. Because IL-12 arose predominantly from circulating HLA-DR+ cells that lacked B-cell- and monocyte-specific markers, we propose that the slow maturation of IL-12-synthetic capacity in the childhood years can be attributed to deficiencies in the number and/or function of dendritic cells.

It is acknowledged that early childhood is characterized by an increased susceptibility to infectious diseases, and this has been attributed both to immaturity of the immune system at birth and to the sluggish development of immune competence in the postnatal and early childhood years. This vulnerability to infections appears to be particularly pronounced in relation to intracellular pathogens, reflecting functional immaturity of cell-mediated immunity. However, considerable gaps still exist in current understanding of the mechanisms underlying immune maturation in healthy children.

While intrinsic properties of T cells undoubtedly contribute to the immunological immaturity observed in the neonatal period (1, 3, 16, 17, 25), it is also clear that many of the "deficiencies" observed in neonatal immune function are dependent on antigen-presenting cells (APCs) (28, 35). Neonatal T cells proliferate relatively poorly when stimulated with freshly isolated allogeneic neonatal dendritic cells (DC) but proliferate as strongly as adult T cells when stimulated with adult DC (18, 24). Similarly, the reduced capacity of neonatal T cells to produce the key T helper type 1 (Th1) cytokine gamma interferon (IFN-γ) is markedly improved when these T cells are cultured with adult, rather than cord, APCs (10, 34,

35), suggesting that neonatal APCs lack the capacity to deliver important Th1 polarizing signals to T cells.

A number of investigators have therefore examined the capacity of cord blood cells to synthesize interleukin 12 (IL-12), a key Th1-trophic cytokine. While reports suggest that neonatal and adult peripheral blood mononuclear cells (PBMC) synthesize equivalent amounts of IL-12 p40 in response to microbial stimuli (32), secretion of bioactive IL-12 p70 by cord blood cells is generally thought to defective (19, 20), perhaps as a consequence of decreased p40 mRNA stability (20). Furthermore, it appears that neonatal monocyte-derived DC also have a specific defect in IL-12 p35 gene expression, though this can be corrected by the addition of exogenous IFN-γ to cultures (15). The exact nature of the signals required to induce neonatal cells to synthesize adult levels of bioactive IL-12 remains poorly understood at present.

There is also a paucity of knowledge concerning the rate at which IL-12 production develops between infancy and adulthood, an issue with fundamental implications for our understanding of host defense and immunoregulation during childhood. We approached this issue by examining the ontogeny of IL-12 production at various ages between infancy and adulthood. In a cross-sectional study, PBMC were obtained from cord blood, 5-year-old children, 12-year-olds, and adults and were examined for their capacity to synthesize IL-12 p70 in response to microbial stimuli.

* Corresponding author. Mailing address: Division of Cell Biology, Telethon Institute for Child Health Research, P.O. Box 855, West Perth WA 6872, Australia. Phone: 618-9489-7839. Fax: 618-9489-7700. E-mail: johnu@ichr.uwa.edu.au.
† Present address: Department of Molecular Biology, Princeton University, Princeton, NJ 08544.

MATERIALS AND METHODS

Culture medium and reagents. Except where indicated, cells were cultured in RPMI 1640 (Gibco BRL) supplemented with L-glutamine, 10% fetal calf serum
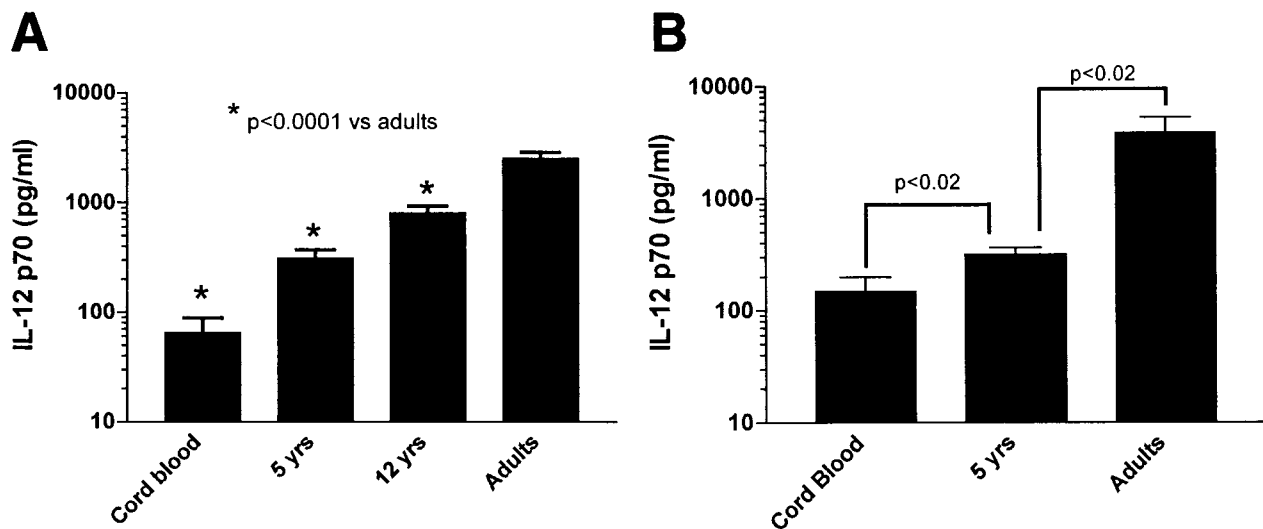
A

B

FIG. 1. Age-related changes in IL-12 p70 synthesis. PBMC were obtained from neonates ($n = 30$), 5-year-old children ($n = 29$), 12-year-olds ($n = 55$), and adults ($n = 12$). After 24 h of stimulation, IL-12 p70 levels were determined by ELISA. Values are means $\pm$ SE. (A) Cells were primed with IFN-γ (10 ng/ml) for 3 h prior to addition of LPS (10 ng/ml). (B) Cells were primed with IFN-γ (10 ng/ml) for 3 h prior to addition of heat-killed *S. aureus* (Pansorbin 0.05%). The statistical significance of comparisons between groups was assessed by an unpaired Student's *t* test.

(Trace BioSciences, Sydney, Australia), 2-mercaptoethanol (4×10^{-5} M; Sigma), and gentamicin (Pharmacia), referred to below as complete medium. The following were purchased: recombinant human IFN-γ (Pharmingen), IL-4 (Pharmingen), *Escherichia coli* lipopolysaccharide (LPS) (serotype O55:B5; Sigma), and heat-killed *Staphylococcus aureus* strain Cowan I (SAC; obtained from Calbiochem under the brand name Pansorbin). Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) was a gift from Glaxo, Geneva, Switzerland.

Subjects. Heparinized cord blood was obtained from the placentas of normal full-term deliveries following elective cesarean section at King Edward Memorial Hospital, Perth, Australia. Recruitment for the study was from antenatal clinics at the King Edward Hospital and from nearby private obstetric practices. Blood samples from healthy 5- and 12-year-old children were obtained from subjects taking part in a community-based, prospective birth cohort study of normal pregnancy. Adult blood samples were from healthy laboratory volunteers. This study was carried out with the approval of the Ethics Committee, Princess Margaret and King Edward Memorial Hospitals, and the parents or guardians of all children provided written consent.

Cell preparation and culture. Mononuclear cells (PBMC) were isolated by density gradient centrifugation and cryopreserved in liquid nitrogen, as previously described (21). Previous studies from our laboratory and elsewhere have demonstrated that this procedure does not distort cellular immune responses (4, 21). In particular, the capacities of both cord blood and adult PBMC to synthesize IL-12 are not influenced by cryopreservation (our unpublished data).

For functional studies, PBMC that had been cryopreserved were thawed and resuspended at 10^6 viable cells/ml in complete medium. Cells were primed with IFN-γ (10 ng/ml) for 3 h prior to addition of either LPS (1 to 100 ng/ml) or SAC (0.01 to 0.05%). These concentrations were chosen based on preliminary dose-response experiments. After culture at 37°C under 5% CO$_2$ for 24 h, supernatants were collected and stored at -20°C for analysis of cytokine production by enzyme-linked immunosorbent assay (ELISA). In preliminary experiments with adult and cord blood PBMC, we determined that IL-12 p70 levels reached a plateau at 24 h after stimulation and did not increase further at 48 h.

Generation of DC from peripheral blood. DC were generated from adult or cord monocytes as described elsewhere (29), with minor modifications. In our initial experiments, adherence proved to be an inefficient method for isolation of monocytes from cord blood samples, because of inconsistent adherence and contamination of samples with fetal nucleated erythrocytes. Depletion of fetal erythrocytes by use of glycophorin A antibodies and immunomagnetic beads has been described previously (13), so this was incorporated into a method for monocyte isolation by negative selection. By using antibodies against CD2, CD7, CD16, CD56, CD19, and glycophorin A, followed by anti-mouse immunomagnetic beads (Dynal), a population containing >90% CD14$^+$ cells was obtained. These monocytes were then cultured in complete medium supplemented with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). Every 2 or 3 days, fresh medium, GM-CSF, and IL-4 were added. After 7 days of culture, nonadherent cells corresponding to the DC-enriched fraction were harvested, washed, and used for subsequent experiments. This DC-enriched fraction contained >90% DC as determined by morphology and flow cytometry (CD1a staining) and by the ability of these cells to stimulate a vigorous allogeneic mixed leukocyte reaction.

Flow cytometric analysis. For immunophenotyping, cells were washed in phosphate-buffered saline supplemented with 0.1% sodium azide and 1% normal human serum and were incubated for 30 min on ice with one of the following monoclonal antibodies (MAbs) (all obtained from BD Pharmingen): fluorescein isothiocyanate (FITC)-conjugated CD1a, FITC-conjugated CD14, phycoerythrin (PE)-conjugated CD80, PE-conjugated CD86, and peridinin chlorophyll protein-conjugated HLA-DR. Cells were also similarly incubated with corresponding isotype-matched control MAbs. Circulating DC were enumerated as "lineage-negative," HLA-DR$^+$ cells by using a commercial kit containing FITC-conjugated CD3, CD14, CD16, CD19, CD20, and CD56, PE-conjugated CD123, PerCP-conjugated HLA-DR, and CD11c APC (BD Pharmingen). Analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson).

Detection of IL-12 and IL-10 by ELISA. Levels of IL-12 and IL-10 in culture supernatant were determined by specific ELISAs. IL-12 p70 protein was measured by an in-house ELISA, using a rat anti-human IL-12 p70 MAb (clone 20C2; Pharmingen) for capture and biotinylated mouse anti-human IL-12 p40/p70 (clone C8.6; Pharmingen) for detection. The standard curve was generated using serial dilutions of recombinant IL-12 p70 (Hoffmann-La Roche); the limit of detection was 15 pg/ml. IL-10 protein was measured as previously described (31). The limit of detection was 4 pg/ml.

Statistical analysis. Data were expressed as means $\pm$ standard errors (SE), and the statistical significance of comparisons was assessed by either a paired or an unpaired *t* test as appropriate.

RESULTS

Age-related changes in capacity to synthesize IL-12 p70. We first examined the synthesis of bioactive IL-12 p70 by PBMC obtained from healthy subjects of various ages, ranging from neonates to adults. Following IFN-γ priming, LPS-stimulated PBMC from adults released substantial amounts of IL-12 p70 ($1,922 \pm 358$ pg/ml), in accordance with previous reports (Fig. 1A). By contrast, and in line with our predictions, neonatal PBMC that had been stimulated in the same manner released very little IL-12 (64.1 ± 22.9 pg/ml). What was most surprising
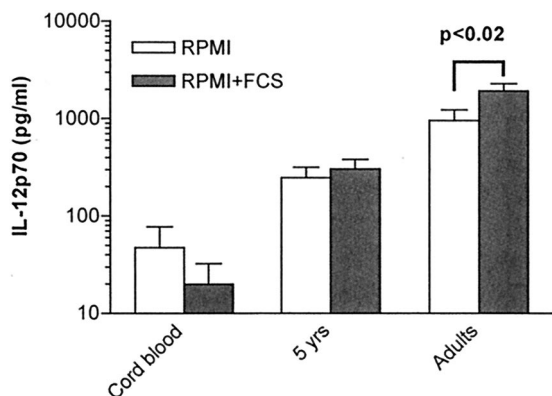
FIG. 2. Effects of serum factors on IL-12 p70 synthesis. PBMC were cultured in RPMI either alone or supplemented with 10% FCS and were stimulated with IFN-γ (10 ng/ml) for 3 h prior to addition of LPS (10 ng/ml). After 24 h, IL-12 p70 levels were determined by ELISA. Values are means ± SE for at least nine individuals from each age group. The statistical significance of comparisons was assessed by a paired t test.

Depleted Populations

FIG. 3. Cellular source of IL-12 p70. Monocytes, B cells, NK cells, and class II MHC^{+} cells were depleted from adult PBMC, by using immunomagnetic beads specific for CD14, CD19, CD56, or HLA-DR, to less than 2% of the starting populations. Cells were primed with IFN-γ (10 ng/ml) for 3 h prior to addition of LPS (10 ng/ml). After 24 h of stimulation, IL-12 p70 levels were determined by ELISA, and the results were compared to those for undepleted PBMC ($n = 5$). Only HLA-DR depletion led to a significant reduction in IL-12 p70 synthesis.

was the slowness with which IL-12-synthetic capacity developed during the intervening years. The levels of IL-12 synthesized by PBMC from both 5-year-old children (311.9 ± 58.4 pg/ml) and 12-year-olds (778.7 ± 123.5 pg/ml) were still substantially below those seen in adults. The data shown were obtained with an LPS concentration of 10 ng/ml; a similar pattern of results was observed with LPS concentrations from 1 to 100 ng/ml (data not shown).

We next examined the ability of heat-killed SAC to activate PBMC, in order to determine whether the reduced capacity to synthesize IL-12 in childhood might be stimulus specific. As with LPS, SAC also induced the synthesis of high levels of IL-12 by adult PBMC, whereas PBMC from neonates and children released significantly smaller amounts of IL-12 (Fig. 1B). The data shown in Fig. 1B were obtained with a SAC concentration of 0.05%; a similar pattern of results was observed with a concentration of 0.01% (data not shown). Given that LPS and SAC activate cells via distinct Toll-like receptors (TLR4 and TLR2, respectively), it thus appeared unlikely that the reduced levels of IL-12 synthesis observed in the two pediatric populations can be attributed to selective impairment of signaling through either TLR2 or TLR4.

IL-12 p70 synthesis in children: effect of serum. Neonatal leukocytes have also been shown to synthesize less tumor necrosis factor alpha (TNF-α) than adult cells in response to LPS (8). However, this deficit in TNF-α release can be partially overcome by the addition of adult serum to neonatal cells cultured in medium alone, suggesting that neonatal cells do have an intrinsic capacity to respond to endotoxin, provided that a key factor(s) in serum is present in culture. Accordingly, we examined whether sera from various sources were also able to augment IL-12 synthesis by PBMC from neonates and children. Whereas addition of fetal calf serum (FCS) to RPMI significantly boosted IL-12 p70 release by adult PBMC stimulated with endotoxin, FCS had no such effect when added to PBMC from neonates and children (Fig. 2). Similarly, addition of normal adult AB serum to RPMI also enhanced IL-12 p70 release by adult PBMC but had no effect on IL-12 p70 release
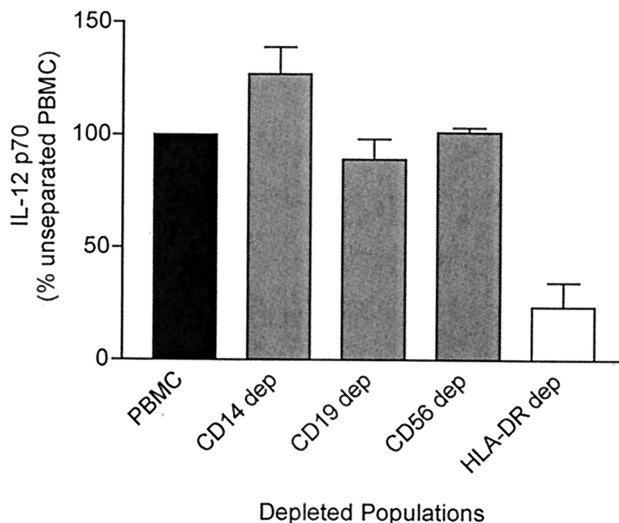
by PBMC from the two pediatric populations (data not shown). When adult and cord blood PBMC were cultured in the defined serum-free medium AIM-V (Gibco), IL-12 p70 levels were greater than those observed with RPMI alone but still well below those observed when PBMC were cultured in RPMI supplemented with either FCS or normal adult AB serum (data not shown). These findings suggest that the reduced capacity for IL-12 synthesis in childhood stems from a reduced capacity of LPS-serum protein complexes to specifically activate IL-12 genes rather than from an inability to respond to endotoxin per se.

Cellular source of IL-12. Because IL-12 can be produced by a variety of circulating mononuclear cells, we sought to determine whether the absence of particular cell populations in cord blood might explain the reduced capacity for IL-12 p70 synthesis. Early reports suggested that IL-12 production by adult PBMC could largely be attributed to major histocompatibility complex (MHC) class II-positive cells, particularly B cells and monocytes (9, 12). While efficient depletion of HLA-DR^{+} cells from adult PBMC by using immunomagnetic beads inhibited IL-12 production, depletion of the CD14^{+}, CD19^{+}, or CD56^{+} populations from adult PBMC did not reduce IL-12 synthesis in response to LPS and IFN-γ stimulation (Fig. 3). In some subjects, depletion of CD14^{+} cells actually increased IL-12 production, suggesting that monocytes might inhibit IL-12 production under some circumstances. However, there was no evidence that neonatal monocytes had a similar suppressive effect on IL-12. Depletion of CD14^{+} cells from cord blood PBMC did not enhance IL-12 synthesis following LPS and IFN-γ stimulation, thereby excluding a suppressive effect of neonatal monocytes as an explanation for the reduced IL-12
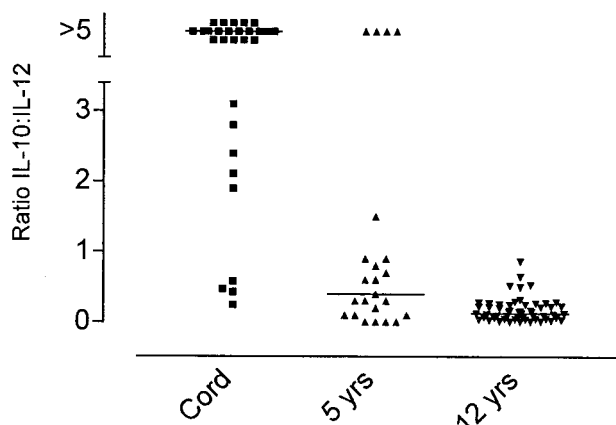
FIG. 4. Age-related changes in synthesis of IL-10 and IL-12. PBMC from neonates, 5-year-old children, and adults were stimulated with LPS plus IFN-γ. After 24 h, IL-10 and IL-12 p70 concentrations were determined by ELISA. Data are shown as ratios of IL-10 to IL-12.

FIG. 5. Monocyte-derived DC from neonates and adults have similar capacities to synthesize IL-12 p70. DC were generated from adult or neonatal CD14^{+} monocytes, by using GM-CSF and IL-4, as described in Materials and Methods and were primed with IFN-γ (10 ng/ml) for 3 h prior to addition of LPS (10 ng/ml). After 24 h, IL-12 p70 levels were determined by ELISA. Data are shown as means ± SE.

synthesis. (Because of limitations in numbers of available cells, it was not possible to conduct these experiments using cells from 5-year-olds and 12-year-olds).

These experiments suggested, much as expected, that the major sources of IL-12 in adult peripheral blood are HLA-DR^{+} cells that lack CD14 and CD19 and are thus putative DC. We thus enumerated DC (lineage negative, HLA-DR[+]) in adult and cord PBMC. Cord blood DC numbers (0.41% ± 0.26% of PBMC) were reduced compared to adult blood DC numbers (0.82% ± 0.24% of PBMC [data not shown]), as in a recent report (33). However, this modest reduction in DC numbers did not appear sufficient on its own to explain the marked reduction in IL-12-synthetic capacity observed in cord blood cells, suggesting that additional deficits in DC maturation were responsible.

Relative production of IL-10 and IL-12 is altered in neonates. We next examined IL-10 synthesis by PBMC from the different age groups, given that this cytokine is an important regulator of IL-12 production. Data shown in Fig. 4 are expressed as IL-10/IL-12 ratios. The response of cord blood PBMC to LPS–IFN-γ stimulation was characterized by production of significant amounts of IL-10 and little if any IL-12, such that the IL-10/IL-12 ratios were nearly always >2 (Fig. 4). In contrast, the reverse was seen in 5-year-olds, 12-year-olds, and adults, where IL-12 production was greater than IL-10 production and the IL-10/IL-12 ratios were nearly always <2.

IL-12 synthesis by monocyte-derived DC. We next asked whether the impaired capacity to synthesize IL-12 is an intrinsic property of circulating APCs in childhood, or whether these cells can be induced to mature in response to cytokines. Accordingly, purified CD14^{+} monocytes from adult and cord blood were induced to undergo DC differentiation in vitro by use of GM-CSF and IL-4 for 7 days. (Because of limitations in numbers of available cells, it was not possible to conduct these experiments using cells from 5-year-old children and 12-year-olds). The yields of DC were similar in both adult and cord blood experiments. Both monocyte-derived DC populations expressed HLA-DR, CD80, and CD86 to similar extents and displayed similar APC function, as determined by allogeneic
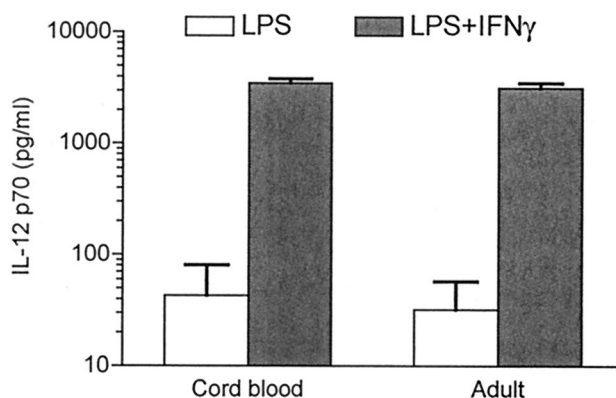
MLR using adult T cells as the responder population (data not shown). Importantly, the two DC populations released equivalent amounts of IL-12 p70 following stimulation with either LPS alone or LPS plus IFN-γ (Fig. 5). Thus, it appears that the reduced ability of neonatal mononuclear cells to synthesize bioactive IL-12 can be overcome following short-term exposure to maturation signals such as GM-CSF and IL-4.

DISCUSSION

Childhood clearly represents a critical period in the development of immune responsiveness, with important implications for the development of both infectious and noninfectious disorders. In order to gain insight into the mechanisms responsible for impaired Th1 responses in childhood, we examined the ontogeny of IL-12 in a population of healthy individuals. The salient findings to emerge were that the capacity of PBMC to synthesize bioactive IL-12 p70 was markedly impaired at birth, that it matured surprisingly slowly during childhood, and that adult levels of IL-12 p70 synthesis were not acquired until after the age of 12 years. This pattern of reduced IL-12 production was observed in response to stimulation with two microbial products that initiate IL-12 gene expression via distinct mechanisms.

A variety of in vivo and in vitro studies have demonstrated that Th1 lineage development is generally deficient in the early postnatal period (5, 6, 27, 36), particularly the ability to develop stable Th1 memory (1, 2). However, it is also clear that strong neonatal Th1 responses can be elicited by the provision of exogenous IL-12 (11, 37) or by the use of adult, rather than neonatal, APCs (34, 35), suggesting that the maturity of APC populations is a key rate-limiting step in the postnatal development of Th1 function. This notion is supported by the finding that antigen challenge in the context of potent adjuvants that are known to up-regulate APC function allows neonatal animals to mount mature Th1 responses (14, 23, 26). Similarly, variations in the immune responses that follow neonatal immunization in humans are likely to be secondary to the differing adjuvant properties of particular vaccine antigens and their

effects on accessory cell populations. Whereas mycobacterial vaccines with intrinsic adjuvant properties can induce vigorous IFN-γ responses in many human infants (22), it is clear that antigen-specific responses to diphtheria, tetanus, and pertussis vaccination in infancy are Th2 skewed (31). It therefore appears that the functional deficiencies of neonatal APCs are not intrinsic to these cells, as they can be induced to mature to adult-equivalent functional competence via the provision of appropriate microenvironmental signals. As shown in Fig. 5, the relative inability of neonatal cells to synthesize IL-12 p70 can be readily overcome by the provision of cytokine signals that induce DC differentiation in vitro. Whereas the development of maximal IL-12-synthetic capacity takes many years in vivo, this can be compressed into just a few days in vitro by using cytokines such as GM-CSF and IL-4.

Previous studies of in vitro IL-12 production in the neonatal period have yielded somewhat conflicting results, though this can be partially explained by the fact that some investigators have measured IL-12 p40 rather than the bioactive p70 heterodimer. Those investigators who have focused on IL-12 p70 synthesis have generally concluded that production of the bioactive heterodimer in the neonatal period is reduced following short-term culture (19, 20).

The mechanisms underlying the reduced capacity of neonatal cells to produce bioactive IL-12 are multiple. Lee et al. showed reduced stability of p40 transcripts in cord blood PBMC (20), whereas Goriely et al. demonstrated that neonatal monocyte-derived DC display a marked inhibition of IL-12 p35 gene expression compared to adult DC (15). Importantly, addition of IFN-γ to neonatal DC restored both p35 and IL-12 p70 synthesis to adult levels (15). While our findings with respect to neonatal monocyte derived DC are very similar (Fig. 5), it is also clear that circulating cord mononuclear cells produce very little IL-12 p70, even in the presence of IFN-γ (Fig. 1), and that IFN-γ responsiveness develops only during cytokine-driven differentiation of monocytes to DC. Given that cord blood APC populations appear to express the IFN-γ receptor (39), we suspect that this lack of IFN-γ responsiveness exhibited by cord blood PBMC reflects changes in intracellular signaling in response to IFN-γ. While it is possible that the reduced synthesis of IL-12 p70 observed in neonates can be partly attributed to concomitant IL-10 production and resulting high IL-10/IL-12 ratios (Fig. 4), this does not appear to be a satisfactory explanation for the relatively slow development of IL-12-synthetic capacity observed in later childhood and adolescence (Fig. 4).

While IL-12 can be produced by a variety of circulating mononuclear cells, including DC, monocytes, B cells, and NK cells, our depletion experiments and the work of others suggest that the major source of IL-12 in adult peripheral blood is HLA-DR+ cells that lack CD14, CD19, and CD56 and are thus putative DC. However, it appears that the modest reduction in cord blood HLA-DR+, lineage-negative cell numbers reported by others (33), and confirmed in our present study, is insufficient on its own to explain the marked reduction in IL-12-synthetic capacity observed in cord blood cells. Neonatal DC appear immature compared to their adult counterparts, with reduced expression of key surface molecules and reduced capacity for antigen presentation (18, 33). Therefore, the immaturity of cord blood DC appears to be the most likely expla-

nation for reduced IL-12 p70 production by cord PBMC, though this will be need to be confirmed in future studies of highly purified cord blood DC.

The capacity to synthesize adult levels of IL-12 does not appear to develop until well into adolescence, and the factors that regulate the ontogeny of IL-12 remain poorly defined. Variations in the rate at which individuals develop the capacity to synthesize IL-12 are almost certainly determined both by variations in the IL-12 genes themselves and by variations in the pattern of exposure to microbial stimuli during childhood. Other than cord blood studies, there have been no studies to our knowledge of IL-12 production by healthy children in the postnatal period. Bont et al. recently examined IL-12 release by PBMC from young infants with severe respiratory syncytial virus (RSV) infection (7). IL-12 production was generally lower than 100 pg/ml following LPS-plus-IFN-γ stimulation, though it was not clear whether the levels of IL-12 produced were influenced by the RSV infection, as the experiments were not repeated when the children had recovered from the infection. There is clearly a need, therefore, for longitudinal studies of IL-12 in children that examine the effects of defined infections or vaccinations and IL-12 polymorphisms on the production of this important cytokine, and also for studies of the relationship between the kinetics of postnatal development of IL-12 and the capacity to respond to vaccine or infectious challenge.

In this context, we have recently examined IFN-γ ontogeny in a cohort of children and found evidence of an inverse relationship between susceptibility to RSV infection and the kinetics of postnatal development of the capacity of PBMC to secrete IFN-γ (30). Of further interest, we demonstrated that for children immunized at 2, 4, and 6 months of age, in vitro IFN-γ responses to tetanus toxoid varied markedly in stability over the ensuing year, with vaccine-specific IFN-γ reactivity being lost in a substantial subset (30). Such instability of Th1 function in the early postnatal period is similar to that reported for the mouse (1). Significantly, it is becoming clear that IL-12 is not only required during the initial phases of Th1 polarization but is also required to maintain the efficiency of the IFN-γ transcription machinery in Th1 effector cells (38). Because Th1 memory cells lose their capacity to produce IFN-γ in the absence of sufficient IL-12 (38), we speculate that the low and variable levels of IL-12 production demonstrated here may provide an explanation for our earlier findings on the instability of vaccine-specific Th1 memory in infants (30). Future studies will address this very important issue.

REFERENCES

1. **Adkins, B., Y. Bu, and P. Guevara.** 2001. The generation of Th memory in neonates versus adults: prolonged primary Th2 effector function and impaired development of Th1 memory effector function in murine neonates. J. Immunol. **166:**918–925.
2. **Adkins, B., and R. Q. Du.** 1998. Newborn mice develop balanced Th1/Th2 primary effector responses in vivo but are biased to Th2 secondary responses. J. Immunol. **160:**4217–4224.
3. **Ansart-Pirenne, H., N. Soulimani, E. Tartour, P. Blot, and G. Sterkers.** 1999. Defective IL2 gene expression in newborn is accompanied with impaired tyrosine-phosphorylation in T cells. Pediatr. Res. **45:**409–413.

4. **Ausiello, C. M., R. Lande, F. Urbani, A. la Sala, P. Stefanelli, S. Salmaso, P. tMastrantonio, and A. Cassone.** 1999. Cell-mediated immune responses in four-year-old children after primary immunization with acellular pertussis vaccines. Infect. Immun. **67:**4064–4071.

5. **Barrios, C., C. Brandt, M. Berney, P. H. Lambert, and C. A. Siegrist.** 1996. Partial correction of the TH2/TH1 imbalance in neonatal murine responses to vaccine antigens through selective adjuvant effects. Eur. J. Immunol. **26:**2666–2670.

6. **Barrios, C., P. Brawand, M. Berney, C. Brandt, P. H. Lambert, and C. A. Siegrist.** 1996. Neonatal and early life immune responses to various forms of vaccine antigens qualitatively differ from adult responses: predominance of a Th2-biased pattern which persists after adult boosting. Eur. J. Immunol. **26:**1489–1496.

7. **Bont, L., A. Kavelaars, C. J. Heijnen, A. J. van Vught, and J. L. Kimpen.** 2000. Monocyte interleukin-12 production is inversely related to duration of respiratory failure in respiratory syncytial virus bronchiolitis. J. Infect. Dis. **181:**1772–1775.

8. **Cohen, L., A. Haziot, D. R. Shen, X. Y. Lin, C. Sia, R. Harper, J. Silver, and S. M. Goyert.** 1995. CD14-independent responses to LPS require a serum factor that is absent from neonates. J. Immunol. **155:**5337–5342.

9. **D'Andrea, A., M. Rengaraju, N. M. Valiante, J. Chehimi, M. Kubin, M. Aste, S. H. Chan, M. Kobayashi, D. Young, E. Nickbarg, et al.** 1992. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J. Exp. Med. **176:**1387–1398.

10. **Delespesse, G., L. P. Yang, Y. Ohshima, C. Demeure, U. Shu, D. G. Byun, and M. Sarfati.** 1998. Maturation of human neonatal CD4$^+$ and CD8$^+$ T lymphocytes into Th1/Th2 effectors. Vaccine **16:**1415–1419.

11. **Donckier, V., V. Flamand, F. Desalle, M. L. Vanderhaeghen, M. de Veerman, K. Thielemans, D. Abramowicz, and M. Goldman.** 1998. IL-12 prevents neonatal induction of transplantation tolerance in mice. Eur. J. Immunol. **28:**1426–1430.

12. **Elenkov, I. J., E. Webster, D. A. Papanicolaou, T. A. Fleisher, G. P. Chrousos, and R. L. Wilder.** 1998. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. J. Immunol. **161:**2586–2593.

13. **Elliott, S. R., P. J. Macardle, and H. Zola.** 1998. Removal of erythroid cells from umbilical cord blood mononuclear cell preparations using magnetic beads and a monoclonal antibody against glycophorin A. J. Immunol. Methods **217:**121–130.

14. **Forsthuber, T., H. C. Yip, and P. V. Lehmann.** 1996. Induction of TH1 and TH2 immunity in neonatal mice. Science **271:**1728–1730.

15. **Goriely, S., B. Vincart, P. Stordeur, J. Vekemans, F. Willems, M. Goldman, and D. De Wit.** 2001. Deficient IL-12 (p35) gene expression by dendritic cells derived from neonatal monocytes. J. Immunol. **166:**2141–2146.

16. **Harris, D. T., M. J. Schumacher, J. Locascio, F. J. Besencon, G. B. Olson, D. DeLuca, L. Shenker, J. Bard, and E. A. Boyse.** 1992. Phenotypic and functional immaturity of human umbilical cord blood T lymphocytes. Proc. Natl. Acad. Sci. USA **89:**10006–10010.

17. **Holt, P. G., J. B. Clough, B. J. Holt, M. J. Baron-Hay, A. H. Rose, B. W. Robinson, and W. R. Thomas.** 1992. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. Clin. Exp. Allergy **22:**1093–1099.

18. **Hunt, D. W., H. I. Huppertz, H. J. Jiang, and R. E. Petty.** 1994. Studies of human cord blood dendritic cells: evidence for functional immaturity. Blood **84:**4333–4343.

19. **Joyner, J. L., N. H. Augustine, K. A. Taylor, T. R. La Pine, and H. R. Hill.** 2000. Effects of group B streptococci on cord and adult mononuclear cell interleukin-12 and interferon-gamma mRNA accumulation and protein secretion. J. Infect. Dis. **182:**974–977.

20. **Lee, S. M., Y. Suen, L. Chang, V. Bruner, J. Qian, J. Indes, E. Knoppel, C. van de Ven, and M. S. Cairo.** 1996. Decreased interleukin-12 (IL-12) from activated cord versus adult peripheral blood mononuclear cells and upregulation of interferon-gamma, natural killer, and lymphokine-activated killer activity by IL-12 in cord blood mononuclear cells. Blood **88:**945–954.

21. **Macaubas, C., P. D. Sly, P. Burton, K. Tiller, A. Yabuhara, B. J. Holt, T. B. Smallacombe, G. Kendall, M. C. Jenmalm, and P. G. Holt.** 1999. Regulation of T-helper cell responses to inhalant allergen during early childhood. Clin. Exp. Allergy **29:**1223–1231.

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

23. **Martinez, X., C. Brandt, F. Saddallah, C. Tougne, C. Barrios, F. Wild, G. Dougan, P. H. Lambert, and C. A. Siegrist.** 1997. DNA immunization circumvents deficient induction of T helper type 1 and cytotoxic T lymphocyte responses in neonates and during early life. Proc. Natl. Acad. Sci. USA **94:**8726–8731.

24. **Matthews, N. C., M. Wadhwa, C. Bird, F. E. Borras, and C. V. Navarrete.** 2000. Sustained expression of CD154 (CD40L) and proinflammatory cytokine production by alloantigen-stimulated umbilical cord blood T cells. J. Immunol. **164:**6206–6212.

25. **Melvin, A. J., M. E. McGurn, S. J. Bort, C. Gibson, and D. B. Lewis.** 1995. Hypomethylation of the interferon-gamma gene correlates with its expression by primary T-lineage cells. Eur. J. Immunol. **25:**426–430.

26. **Min, B., K. L. Legge, J. C. Caprio, L. Li, R. Gregg, and H. Zaghouani.** 2000. Differential control of neonatal tolerance by antigen dose versus extended exposure and adjuvant. Cell. Immunol. **200:**45–55.

27. **Prescott, S. L., C. Macaubas, B. J. Holt, T. B. Smallacombe, R. Loh, P. D. Sly, and P. G. Holt.** 1998. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J. Immunol. **160:**4730–4737.

28. **Ridge, J. P., E. J. Fuchs, and P. Matzinger.** 1996. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. Science **271:**1723–1726.

29. **Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler.** 1994. Proliferating dendritic cell progenitors in human blood. J. Exp. Med. **180:**83–93.

30. **Rowe, J., C. Macaubas, T. Monger, B. J. Holt, J. Harvey, J. T. Poolman, R. Loh, P. D. Sly, and P. G. Holt.** 2001. Heterogeneity in diphtheria-tetanus-acellular pertussis vaccine-specific cellular immunity during infancy: relationship to variations in the kinetics of postnatal maturation of systemic Th1 function. J. Infect. Dis. **184:**80–88.

31. **Rowe, J., C. Macaubas, T. M. Monger, B. J. Holt, J. Harvey, J. T. Poolman, P. D. Sly, and P. G. Holt.** 2000. Antigen-specific responses to diphtheria-tetanus-acellular pertussis vaccine in human infants are initially Th2 polarized. Infect. Immun. **68:**3873–3877.

32. **Scott, M. E., M. Kubin, and S. Kohl.** 1997. High level interleukin-12 production, but diminished interferon-gamma production, by cord blood mononuclear cells. Pediatr. Res. **41:**547–553.

33. **Sorg, R. V., G. Kogler, and P. Wernet.** 1999. Identification of cord blood dendritic cells as an immature CD11c- population. Blood **93:**2302–2307.

34. **Taylor, S., and Y. J. Bryson.** 1985. Impaired production of gamma-interferon by newborn cells in vitro is due to a functionally immature macrophage. J. Immunol. **134:**1493–1497.

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

36. **Wilson, C. B., J. Westall, L. Johnston, D. B. Lewis, S. K. Dower, and A. R. Alpert.** 1986. Decreased production of interferon-gamma by human neonatal cells. Intrinsic and regulatory deficiencies. J. Clin. Investig. **77:**860–867.

37. **Wu, C. Y., C. Demeure, M. Kiniwa, M. Gately, and G. Delespesse.** 1993. IL-12 induces the production of IFN-γ by neonatal human CD4 T cells. J. Immunol. **151:**1938–1949.

38. **Yap, G., M. Pesin, and A. Sher.** 2000. Cutting edge: IL-12 is required for the maintenance of IFN-γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii.* J. Immunol. **165:**628–631.

39. **Zola, H., M. Fusco, P. J. Macardle, L. Flego, and D. Roberton.** 1995. Expression of cytokine receptors by human cord blood lymphocytes: comparison with adult blood lymphocytes. Pediatr. Res. **38:**397–403.