

# Analysis of T Helper and Antigen-presenting Cell Functions in Cord Blood and Peripheral Blood Leukocytes from Healthy Children of Different Ages

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## Abstract

The development of antigen-specific functional T lymphocyte immunity in infants and children is an area of immunology that needs elucidation. Leukocytes from cord blood (CBL) and from PBL of children of different ages who were in the hospital for minor surgical procedures were compared with PBL from healthy adults for their ability to generate T helper cell (Th) responses assessed by in vitro proliferation and IL-2 production after stimulation with: influenza A virus (FLU); tetanus toxoid (TET); adult allogeneic PBL that were either undepleted (ALLO) or depleted of adherent antigen presenting cells (ALLONW); and PHA. CBL generated Th responses to ALLO, ALLO, and PHA, but not to FLU or TET. PBL from infants between 6 and 13 mo of age responded to ALLO and PHA; none responded to FLU or ALLONW, and two of four responded weakly to TET. PBL from children between 13 and 26 mo of age responded to all stimuli except FLU, to which only one child responded marginally. PBL from children older than 36 mo responded to all stimuli at levels comparable to those of PBL from adults.

The use of undepleted and adherent cell-depleted CBL and PBL from children of different ages as allogeneic stimulators of responses generated by PBL from adults indicated that the antigen presenting function of CBL and PBL from children 13 mo or older are sufficiently developed to present alloantigen, whereas PBL from children younger than 13 mo are not. Therefore, our results indicate that age-dependent differences exist in both T helper and antigen-presenting functions of CBL and PBL from children of different ages. Surprisingly, CBL appear to be more efficient in antigen-presenting function than PBL from children younger than 13 mo. These findings are important for establishing developmental parameters of T helper cell immunity relevant for pediatric infection and transplantation in infants and children. (*J. Clin. Invest.* 1993. 91:2829-2836.)  
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## Introduction

An understanding of the developing immune system is an important area of immunology, because it is during this period

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that the individual: (a) acquires the ability to distinguish self from nonself (1); (b) establishes mechanisms in the peripheral immune system to prevent potential autoimmune reactions (1); and (c) develops and expands clones of lymphocytes with immunologic memory necessary to fight infections (2, 3). Recently, human developmental immunity has assumed particular significance, due to the increase in the number of pediatric cases of AIDS, primarily as a result of mother-to-child transmission of HIV, either by transplacental or perinatal transmission (4, 5). Thus, it has become increasingly important to determine: which components of the immune system are functionally mature at birth; when, during postnatal development, is the human immune system capable of responding to different types of antigenic stimuli, particularly to recall antigens; and when, during development, does the ability to generate immune responses to HIV antigens appear in infected infants. Furthermore, the establishment of a timetable for the development of T cell immunity is needed, because it could permit one to distinguish between a lack of T helper cell (Th)<sup>1</sup> and/or antigen-presenting cell (APC) function due to developmental factors versus HIV-induced defects. Studies have been reported demonstrating that neonatal PBL express high affinity IL-2 receptors and produce near adult levels of IL-2 when maximally stimulated with mitogens, but are severely defective in the production of IFN- $\gamma$  and IL-4 (6-8). Other studies have reported that neonatal T lymphocytes are almost entirely of the naive phenotype (2, 3, 9).

In this study we have assessed in vitro-generated Th responses by cord blood leukocytes (CBL) and by PBL from pediatric donors of different ages to a series of T cell stimuli previously used to analyze Th function in HIV-infected (HIV<sup>+</sup>) (10) and uninfected (HIV<sup>-</sup>) (11) adults. These responses include IL-2 production and proliferation to influenza A virus (FLU), tetanus toxoid (TET), allogeneic PBL depleted of antigen-presenting cells by nylon wool adherence (ALLO), undepleted allogeneic PBL (ALLO), and PHA. This panel of stimuli was chosen because Th responses to FLU, TET, and ALLONW are HLA-self-restricted, and require antigen processing and presentation by autologous APC (11). It is probable that Th responses to FLU and TET also require in vivo priming resulting in the maturation and expansion of T cells expressing memory markers, whereas the response to ALLO does not. In contrast, Th responses to ALLO can use APC from the allogeneic stimulator cells, and the response to PHA is not as dependent on APC as those to specific antigens

1. Abbreviations used in this paper: ALLO, allogeneic peripheral blood leukocytes; ALLONW, allogeneic peripheral blood leukocytes depleted of antigen presenting cells; APC, antigen presenting cells; CBL, cord blood leukocytes; CTLL, cytotoxic T lymphocyte cell line; FLU, influenza A virus; TET, tetanus toxoid; Th, T helper cells.

(11). These stimuli were also selected for study in young children because we have previously reported that: (a) PBL from both adult (10) and pediatric (12) HIV<sup>+</sup> individuals exhibit a spectrum of Th defects to this panel of T cell stimuli that is predictive for AIDS progression in adults (10, 13) and for susceptibility to opportunistic and bacterial infections in children (12); and (b) different patterns of function of the Th pathways are predictive for human solid organ allograft rejection (14). The results of this study will be relevant for assessing Th and antigen presenting cell functions that are required for immunologic maturation in infants, and may be important for vaccination and modulation of immunity in infants and children.

## Methods

*In vitro assay for Th function.* The 24 cord bloods were obtained from neonates that ranged from 28 to 42 wks in gestation (median of 39 wks; 8 of the 24 were < 38 wks). Blood was obtained immediately after delivery by isolating a segment of umbilical cord and performing cordocentesis with a sterile needle. In this manner, ~ 7 ml of newborn venous blood was collected into a tube containing 200 USP units of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ). All cord blood samples were obtained from healthy deliveries. A maximum of 3 ml of whole blood was collected from infants < 27 mo of age (15 patients), and 6 ml was collected from children between 36 mo and 14 yr of age (13 patients) by routine phlebotomy in conjunction with preoperative blood tests. These children had been admitted to Children's National Medical Center for minor surgical procedures, which included inguinal hernia repair, tonsilectomy, myringotomy, and strabismus eye surgery. There was no evidence of concurrent infections in these infants. A protocol and consent form for obtaining this volume of blood from the children, as well as the newborns, was reviewed and approved by the Institutional Review Boards of the Fairfax Hospital, the Children's National Medical Center, and the National Cancer Institute. Due to constraints imposed by the Institutional Review Boards, blood from infants younger than 6 mo of age could not be obtained in quantities sufficient to perform experiments, and accrual of infants for the study was limited to the 13 presented in the report. Blood from adult donors was obtained as a buffy coat derived from donors undergoing plasmapheresis in the Department of Transfusion Medicine, National Institutes of Health.

The mononuclear cell fraction was obtained using lymphocyte separation medium (Organon Teknika Corp., Durham, NC), washed twice in PBS, and the number of viable cells was determined by trypan blue exclusion. CBL and PBL were resuspended at  $3 \times 10^6$ /ml in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 0.5% penicillin and 1% glutamine. For both the IL-2 production and the proliferation assays, 0.1 ml of the lymphocyte suspension was added per well in triplicate to 96-well flat bottom microtiter tissue culture plates (Costar Corp., Cambridge, MA). The CBL and PBL were either unstimulated or were stimulated with: (a) influenza A/Bangkok RX73 (H3N2) (FLU) 1:500 final dilution (10, 11); (b) TET at 40 flocculation units/ml final concentration (Massachusetts Department of Health, Boston, MA); (c)  $2 \times 10^5$ /well of 50 Gy irradiated allogeneic stimulator cells consisting of pooled PBL from two unrelated healthy adult donors (ALLO); or (d) PHA (Gibco Laboratories) 1:200 final dilution. Pooled human plasma (5%) was added to each well, and for IL-2 production studies, the anti-IL-2 receptor antibody, monoclonal anti-TAC, (a gift from Dr. T. A. Waldmann, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) was added at the initiation of culture at a concentration of 5  $\mu$ g/ml to block IL-2 consumption (10, 11). Supernatants of the cultures were harvested 7 d after stimulation, and added in triplicate at dilutions of 1:2, 1:4, 1:8, and 1:16 to the IL-2-dependent cytotoxic T lymphocyte (CTLL) cell line in 96-well microtiter plates. The CTLL ( $8 \times 10^3$ /

well) were cultured in the supernatants for 24 h, and pulsed for an additional 18 h with 1  $\mu$ Ci of [<sup>3</sup>H]thymidine (ICN Radiochemicals, Irving, CA). For the proliferation studies, the 96-well cultures were pulsed after 6 d with 1  $\mu$ Ci [<sup>3</sup>H]thymidine and harvested 18 h later. All thymidine pulsed cultures were harvested using a harvesting system (TomTec, Inc., New Haven, CT) and the samples were counted using a plate spectrometer (Pharmacia LKB Biotechnology, Piscataway, NJ). For both the IL-2 and proliferative responses, results are expressed as mean cpm for triplicate wells. Standard errors were consistently < 10% of the mean values. The stimulation index was determined as the ratio of cpm from stimulated cultures to that of unstimulated cultures. Standard titration curves were determined using recombinant IL-2 (Cetus Corp., Emoryville, CA). However, we were not able to calculate accurately IL-2 units because many of the titration curves generated by unresponsive PBL from young children were flat and not parallel to the standard curves or to the curves generated by PBL from control donors. Therefore Figs. 1 and 5 present complete titration curves, and Fig. 2 shows a dot plot at the highest supernatant concentration of 1:2. A response was determined to be positive if the cpm from stimulated wells exceeded the mean background (unstimulated) cpm observed by three standard deviations (10). We have compared and include both IL-2 production and proliferative assays to verify results obtained from a limited number of infants and because of the fact that proliferative responses have been more often reported than IL-2 production data.

*Depletion of adherent cells and CD8<sup>+</sup> cells.* Adherent APC were removed by a two-step process of plastic adherence for 30 min (at 37°C, 7% CO<sub>2</sub>, and 2% human plasma), followed by passage of the cells over a nylon wool column. The depleted cell population contained < 5% monocytes as determined by flow cytometry staining with FITC-conjugated anti-human monocyte/mAb, Leu-M3 (Becton Dickinson, Mountain View, CA) (11, 15). Enrichment of APC was achieved by centrifugation of PBL over a Septracel® gradient (Septracel Corp., Oklahoma City, OK) at 1,500 rpm for 20 min at 22°C. The top band was removed, washed, and irradiated with 50 Gy before use as APC. This fraction contained 90–96% monocytes as determined by flow staining with Leu-M3 (data not shown).

CD8<sup>+</sup> T cells were negatively selected using the panning technique described elsewhere (7). Cells were first incubated with anti-CD8 (Leu-2a) murine mAb (Becton Dickinson), washed, and incubated for 2 h at 4°C in petri dishes precoated with the IgG fraction of goat anti-mouse IgG (Cappel Laboratories, Malvern, PA). After 2 h, nonadherent cells were collected, washed, and adjusted to the desired concentration. Flow cytometric analysis of panned cells by this technique indicated that they consistently contained < 5% of the depleted population of cells (11, 15). This determination was based on staining of the panned cells with FITC-labeled Leu-2a, Leu-3a, or Leu-M3 mAbs, as well as FITC-labeled F(ab')<sub>2</sub> fragment of IgG fraction of goat anti-mouse IgG antibody (Cappel Laboratories) to detect cells that bound antibody but were not depleted.

Samples of CBL from deliveries of three male and one female (control) infants were stimulated with PHA in RPMI 1640 media containing 20% fetal bovine serum. After 72 h of culture, the chromosomes were stained with Giemsa-trypan, and 150 metaphase spreads were analyzed microscopically for each of the four samples (by an individual who did not know the sex of the infants) for the number of X and Y chromosomes.

## Results

*Analysis of T helper cell function.* T helper cell responses assessed by IL-2 production to FLU, TET, ALLONW, ALLO, and PHA are shown in Fig. 1 for individuals of different ages who are representative of the groups that we have studied. CBL from a neonate responded to ALLONW, ALLO, and PHA, but not to FLU or TET (A). PBL from an 11-mo-old infant re-

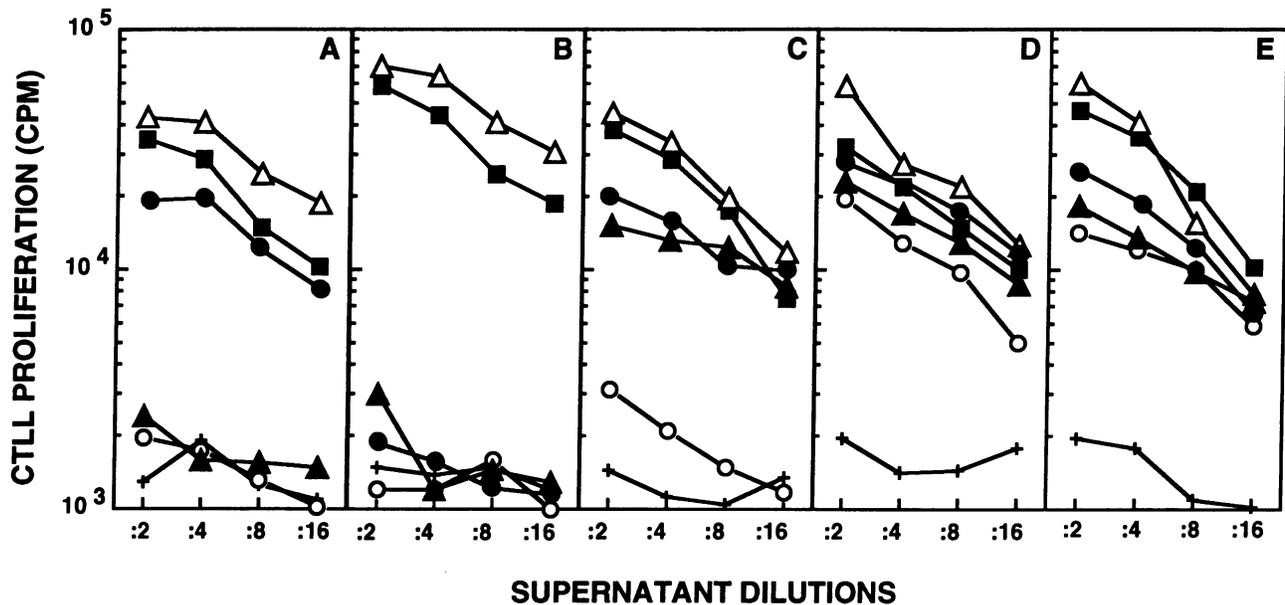


Figure 1. IL-2 production by unstimulated CBL or PBL (+) and by CBL or PBL stimulated with: FLU (○); TET (▲); ALLONW (●); ALLO (■); or PHA (△). (A) CBL; (B) PBL from an 11-mo-old infant; (C) PBL from a 24-mo-old child; (D) from a 6-yr-old child; (E) PBL from an adult. The figure illustrates the IL-2 titration curves of different individuals whose data are representative of their respective groups.

sponded to ALLO and PHA, but not to FLU, TET, or ALLONW (B). PBL from a 24-mo-old child responded to all stimuli except to FLU (C). PBL from a 6-yr-old child responded to all stimuli (D) at a level comparable to PBL from an adult (E). Thus, some remarkable Th functional differences were observed between adjacent panels in that: (a) CBL responded to ALLONW, whereas PBL for the 11-mo-old infant did not (compare A and B); (b) PBL from the 24-mo-old child responded to TET and ALLONW, whereas PBL from the 11-mo-old infant did not (compare B and C); and (c) PBL from the 6-yr-old child responded to all stimuli, whereas PBL from the 24-mo-old failed to respond to FLU (compare C and D).

The mean cpm for IL-2 production generated by the IL-2-sensitive CTLL cell line (at a culture supernatant dilution of 1:2) by CBL and PBL from all samples tested are summarized as a dot plot in Fig. 2. Similar to the IL-2 titration curves shown for representative individuals in Fig. 1, CBL (from 16–24 samples) responded to ALLONW, ALLO, and PHA, but not to FLU or TET. One CBL sample generated a marginal response to TET. PBL from the four infants 12 mo of age or less responded to ALLO and PHA, but not to FLU or ALLONW. Two of the four samples responded weakly to TET. PBL from the 13–26-mo-old children responded strongly to all stimuli except FLU. PBL from the children 36 mo of age or older generated strong responses to all stimuli, as did PBL from nine adults.

We have also tested Th responses to these five stimuli by proliferation. The cpm data and stimulation indices of representative individuals from the five different age groups are shown in Fig. 3. (These are not the same representative individuals used for illustration in Fig. 1.) As was observed for IL-2 production: (a) CBL responded to ALLONW, ALLO, and PHA, but not to FLU or TET (A); (b) PBL from the 11-mo-old infant responded to ALLO and PHA, but not FLU, TET or ALLONW; (c) PBL from the 26-mo-old child responded

strongly to all stimuli except FLU, for which the response was weak (C); and (d) PBL from the 4-yr-old child responded to all stimuli (D), as did PBL from the adult (E).

The mean stimulation indices for proliferative responses to the five stimuli are shown in Fig. 4, and confirm the data of the three preceding figures. Thus, proliferative responses to FLU were: (a) potent in PBL from children 36 mo of age or older and in adults; (b) weak in children between the ages of 13 and 26 mo; and (c) negative in infants 6–12 mo of age, and in CBL (A). Proliferative responses to TET were: (a) marginal by PBL from infants 6–12 mo of age and negative by CBL; (b) stronger in 13–26-mo-old infants than the response to FLU; and (c) were potent and equivalent to adult responses in children older than 36 mo of age (B). The proliferative responses to ALLONW were potent and indistinguishable in CBL and in PBL from children older than 36 mo and adults. PBL from infants between the ages of 6 and 12 mo were unresponsive, and PBL from 13–26-mo-old infants were weakly responsive to ALLONW (C). Proliferative responses by PBL from all groups were strong to ALLO and PHA, although the responses by CBL and PBL from 6–12-mo-old infants were weaker than those from the older groups (D and E).

To exclude the possibility that our results with CBL were artificial due to maternal lymphocyte contamination of CBL, cytogenetic analysis of metaphase spreads of PHA-stimulated CBL from deliveries of male offspring were performed to determine whether the CBL were contaminated with maternal lymphocytes. All 450 of the CBL metaphases spreads from the three male deliveries examined contained one X and one Y chromosome, indicating that no maternal T cells were detected as a contaminant in the CBL samples. The metaphases of CBL from the control female delivery contained only X chromosomes.

*Analysis of antigen-presenting cell function.* Our finding that CBL were responsive to ALLONW (a Th response that

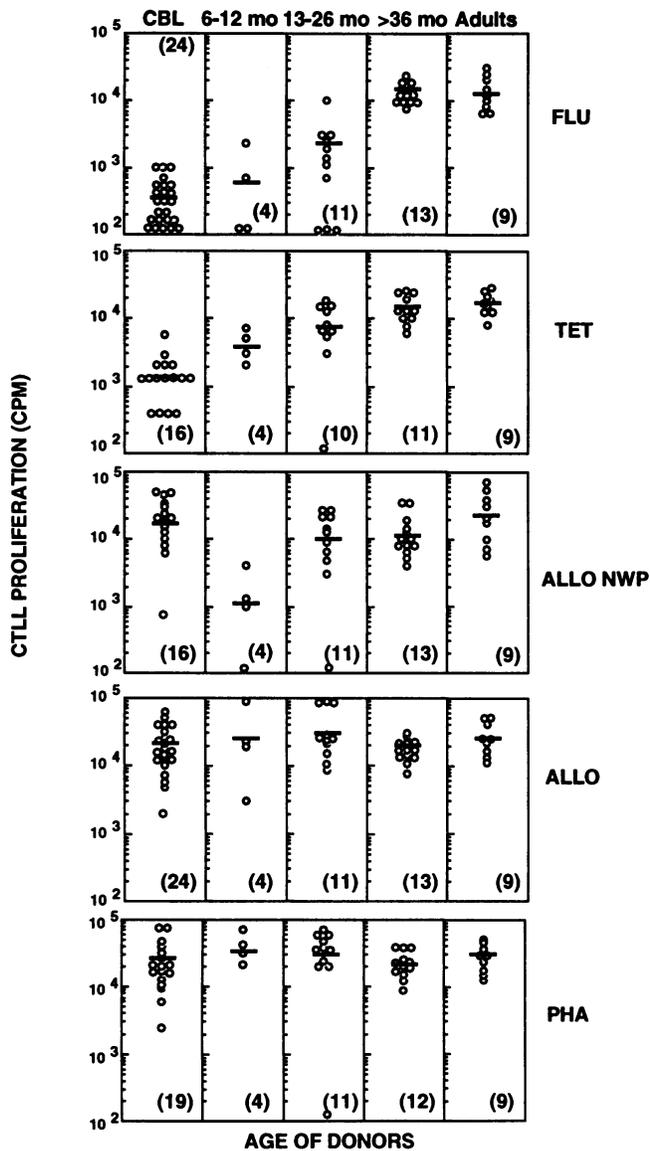


Figure 2. Dot plot and mean values (shown by horizontal bars) of IL-2 produced at a 1:2 supernatant dilution by CBL and PBL from all donors studied. Ages, stimuli, and number of samples tested ( ) are indicated. The mean unstimulated background CPM for the CTLL line was 1472.

uses the same pathway as that for FLU and TET but does not require priming), whereas PBL from infants between 6 and 26 mo of age were either unresponsive or only weakly responsive (Figs. 1-4), raises the possibility that the antigen-presenting function of CBL is more developed than that of infants 6-12 mo of age. To test whether there is an age-related difference in the antigen-presenting function of CBL and PBL of children, we have used their leukocytes as irradiated stimulators of an allogeneic mixed lymphocytes reaction. In this type of experiment, the responder PBL from an unrelated adult donor were either undepleted or were depleted of APC. Under these conditions the allogeneic stimulators used need to provide only the alloantigens when used to stimulate undepleted responder PBL (11). In contrast, the CBL and children's PBL must provide

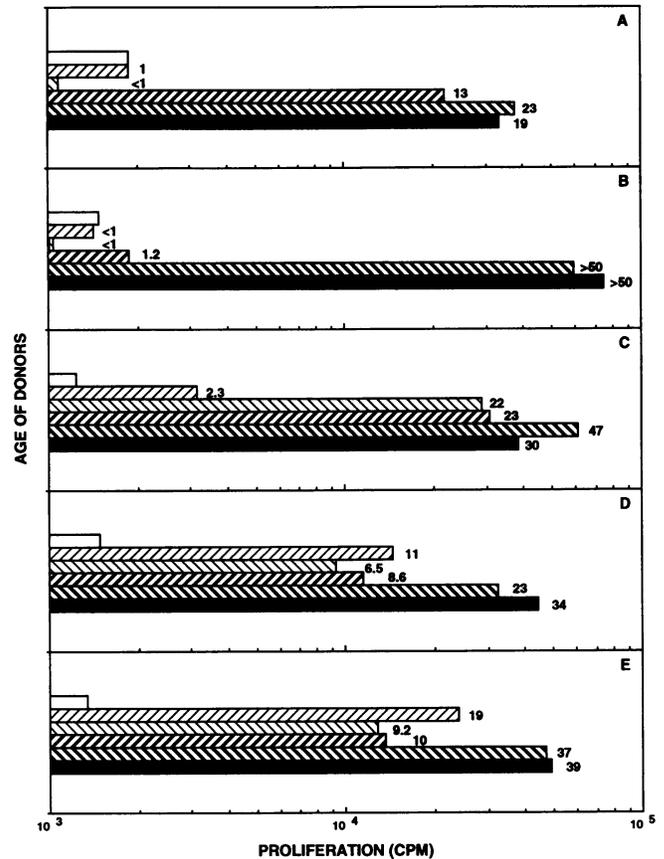


Figure 3. Proliferative responses by unstimulated (□) CBL or PBL, and by CBL or PBL stimulated with: FLU (∅); TET (⊞); ALLO NWP (⊚); ALLO (⊠); or PHA (■). The figure illustrates the proliferation of CBL or PBL from individuals of different ages whose data are representative of the different age groups. (A) CBL; (B) PBL from an 11-mo-old infant; (C) PBL from a 26-mo-old child; (D) PBL from a 4-yr-old child; (E) PBL from an adult. The numbers shown are the stimulation indices.

both alloantigens and antigen-presenting (or accessory) function when used to stimulate responder PBL depleted of APC.

The results of a representative experiment in which undepleted and APC-depleted PBL from an adult were stimulated with CBL or with PBL from children 6 mo, 24 mo, or 6 yr of age, or with adult PBL, all from unrelated donors, are shown in Fig. 5. Both APC-depleted and undepleted adult PBL responded to CBL (A), and to PBL from children 24 mo (C) and 6 yr (D) of age, as well as to adult PBL (E). In contrast, the undepleted adult PBL responded to PBL from an infant 6 mo of age, but the depleted adult PBL did not (B). Furthermore, the response of depleted adult PBL to stimulator PBL from the 24-mo-old child was lower than the response of undepleted adult PBL and lower than the response of depleted adult PBL to stimulator PBL from the 6 yr old. The pattern of response by APC-depleted and undepleted adult responding PBL to allogeneic CBL (four additional donors) and PBL from infants and children ranging from 6 mo to 8 yr of age (six additional donors) is verified in Fig. 6, in which we have summarized the IL-2 titration data by a dot plot for the 1:2 supernatant dilution. These results indicate that CBL (A and B) and PBL from

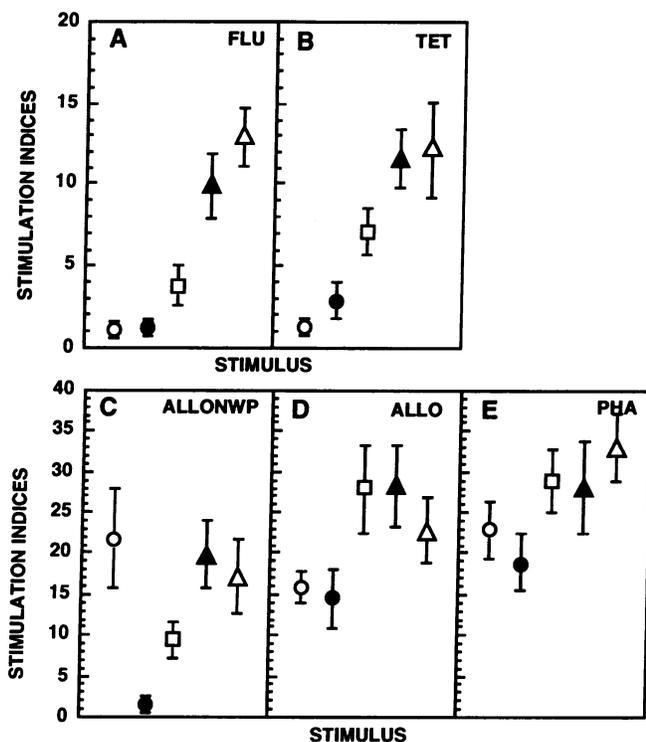


Figure 4. Mean proliferative stimulation indices (and standard errors) of CBL or PBL from donors of different ages stimulated with: (A) FLU; (B) TET; (C) ALLONW; (D) ALLO; and (E) PHA. (○) CBL ( $n = 22$ ); (●) PBL 6–12 mo of age ( $n = 4$ ); (□) PBL 13–26 mo of age ( $n = 9$ ); (▲) PBL > 36 mo ( $n = 13$ ); and (△) adults ( $n = 14$ ).

infants and children older than 13 mo (E and F) can stimulate PBL from an unrelated adult donor, irrespective of whether the responder PBL were depleted of APC. Two exceptions were noted in which APC-depleted PBL from an 18- and a 24-mo-

old infant failed to stimulate adult PBL (F). In contrast, PBL from the three younger children (two of whom were 6 mo and the other of whom was 22 mo of age) were capable of stimulating only adult PBL not depleted of APC (C and D). Taken together, these results indicate something unique about allogeneic mixed lymphocyte response to PBL from infants younger than 13 mo of age that is not seen in either CBL or in PBL from older infants and children. Our data suggest that this difference is due to an APC defect observed only in the younger donors, but not in CBL nor usually in PBL from children older than one year. However, two children 18 and 22 mo of age also exhibited defective APC function.

If CBL are mature enough to provide antigen-presenting function to stimulate an allogeneic mixed lymphocyte reaction generated by adult responder PBL, it should be possible to demonstrate that CBL can serve as responder cells in an allogeneic response in which the CBL are also required to provide antigen-presenting (or accessory) function. To test this possibility, CBL were stimulated with irradiated, undepleted (ALLO) or APC-depleted (ALLONW) PBL from an unrelated adult donor. For stimulation with ALLO, APC function can be provided by the adult stimulator cells. For stimulation with ALLONW, APC function has to be provided by the CBL, although the adult PBL provided the alloantigens. If the responding CBL can provide their own functional APC, then the CBL should still respond to adult allogeneic stimulator PBL, irrespective of whether the latter were depleted of adherent cells. The data of Fig. 7 illustrate two independent experiments (A–C and D–F), using leukocytes from two different cord bloods. Thus, both CBL preparations responded equally well to adult ALLO and ALLONW, indicating that the APC contained in the CBL are capable of providing antigen presenting function for allogeneic stimulation. In addition, we depleted the responder CBL of adherent APC and of CD8<sup>+</sup> lymphocytes, and reconstituted antigen presenting function with either irradiated, autologous CBL or with irradiated, allogeneic adult PBL. B and E show that CD8-depleted CBL do not re-

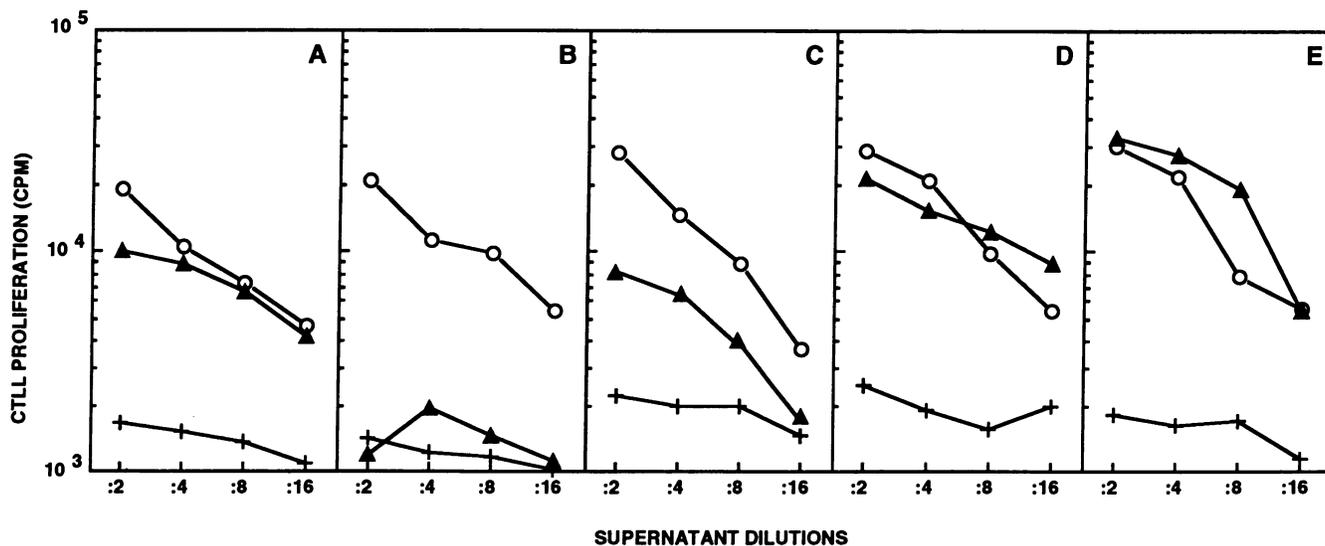


Figure 5. IL-2 production by adult PBL either unstimulated (+) or stimulated with 50 Gy irradiated leukocytes from unrelated donors of different ages. The adult responder PBL were either unseparated (○) or were depleted of APC (▲). Age of stimulator cell donors were: (A) neonate (CBL); (B) 6 mo; (C) 24 mo; (D) 6 yrs; (E) adult.

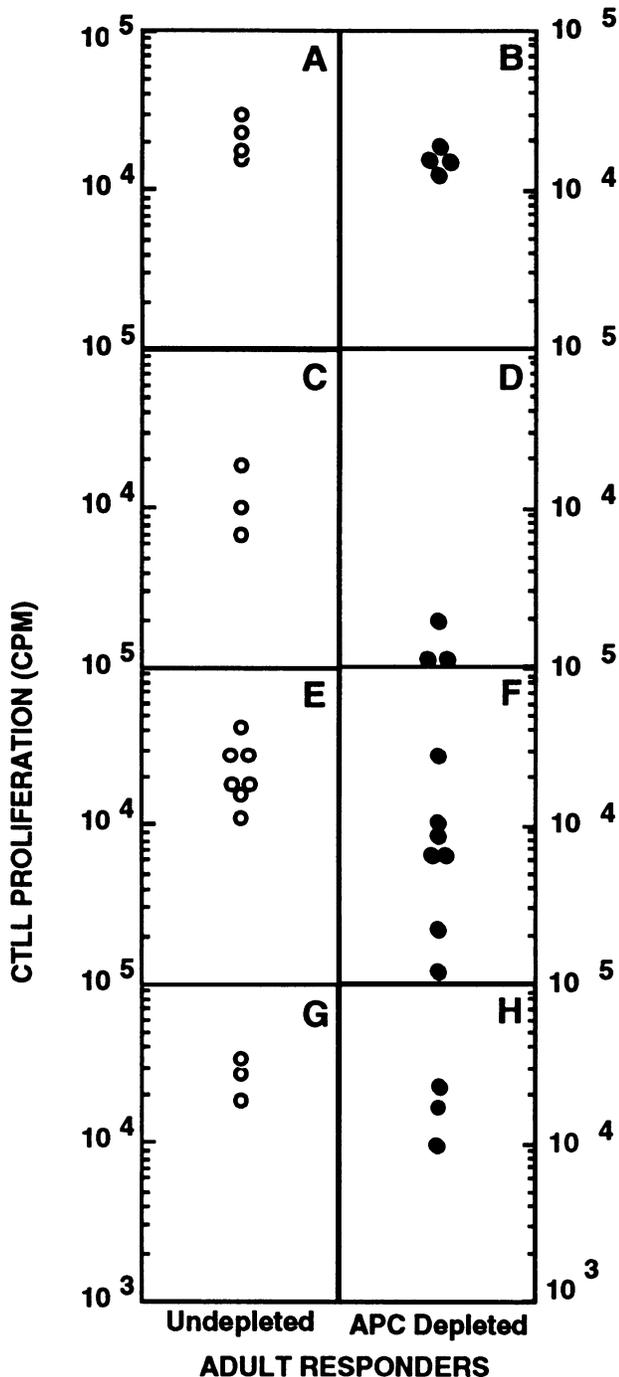


Figure 6. Dot plot of IL-2 produced at a 1:2 supernatant dilution by unseparated (A, C, E, G) or APC-depleted (B, D, F, H) adult PBL to: CBL (four donors) (A, B); PBL from (three) 6-12-mo-old infants (C, D); PBL from (seven) 13-26-mo-old infants (E, F); and PBL from (three) children > 36 mo of age (G, H).

spond to adult ALLONW if the CBL are also depleted of APC. However, CD8-depleted, APC-depleted CBL responded well to adult ALLONW when APC function was provided by either irradiated autologous CBL or by irradiated allogeneic adult PBL (C and F). These results verify that the antigen presenting function of CBL is intact and equivalent to that provided by

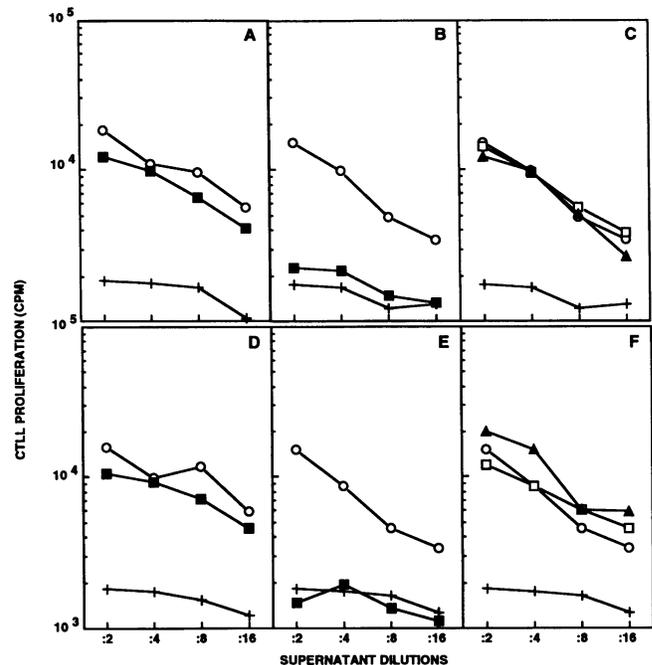


Figure 7. IL-2 production by CBL (A, D) and by CBL depleted of APC and CD8<sup>+</sup> T cells (B, C, E, F). 50 Gy irradiated adult stimulators were: undepleted (○); depleted of APC (■); depleted of APC and CD8<sup>+</sup> cells and reconstituted with adult allogeneic 50 Gy irradiated APC (▲); (C, F) or depleted of APC and CD8<sup>+</sup> cells, and reconstituted with the CBL's autologous 50 Gy APC (□) (C, F). A-C and D-F illustrate two independent experiments.

adult PBL, and suggest that the CD4-mediated, HLA self-restricted component of the allogeneic Th response is functionally intact in cord blood. The clinical experimental protocol for collecting infant blood did not permit us to obtain enough blood to perform these cell depletion experiments using infant PBL as responder cells.

## Discussion

This study has analyzed the development of human T helper lymphocyte and antigen-presenting function by using a panel of stimuli that impose different requirements on the immune system. This particular panel of stimuli was selected because: (a) of our extensive experience in evaluating Th and APC function in HIV<sup>+</sup> individuals (including children) (10, 12); and (b) each of the stimuli tests different components of immunity that contribute to helper cell function (11). For example, both the TET and FLU responses require cells that express memory markers. Our findings that the response to TET occurs before the response to FLU may reflect the fact that infants have been vaccinated with tetanus toxoid. Because infants have not been vaccinated to influenza A virus, the development of memory T cells specific for FLU should depend on natural exposure and would be expected to lag behind the potential to respond to TET.

It is unlikely that the ALLONW response requires priming, but it does require processing by autologous APC (11), and is thereby HLA self-restricted, as are the Th responses to TET

and FLU. Thus, the simultaneous development of Th function to TET and ALLONW by the infants' PBL (see Figs. 1–4) suggests an event that involves maturation of the infants' APC function that has occurred by 13 mo of age. This maturation event could be mediated by cytokine(s), and might involve an increase in the expression of HLA (possibly class II) on APC. Such a mechanism could also account for the unexpected observation that CBL are responsive to ALLONW, whereas PBL from infants less than 13 mo of age are unresponsive. This difference in response to ALLONW that unexpectedly declined after birth could be due to maternal cytokine influences on APC function of CBL, which might elevate HLA antigen expression on APC at the time of birth, but which could be lost with time. Other studies have reported that monocytes from newborns: (a) can process and present antigens as well as monocytes from adults (16); and (b) express surface Ia antigens, produce interleukin 1, and present bacterial antigens as well as do monocytes from adults (17). Such studies have not tested for the possibility of a decline in APC function after birth. The return of Th function in response to ALLONW at ~ 1 yr of age could reflect endogenous APC maturation by the infant. It is unlikely that the response of CBL to ALLONW was due to contaminating maternal PBL, because no female cells were observed in 450 metaphases examined from three male births.

The present study is also important in that it establishes a timetable for the development of T helper cell immune responses to infectious agents. Without the knowledge of this sequence of maturational events, it may not be possible to distinguish between an absence of T helper immunity in infants that is due to lack of cell development versus HIV-induced immune dysfunction. It is also noteworthy that the CBL from 28-wk to full-term deliveries all exhibited similar patterns of immune maturation. This observation suggests that T cell immune potential of 28-wk CBL is as mature for the stimuli tested as that of full-term infants.

Neonatal lymphocytes have been reported to be defective in IFN- $\gamma$  and in IL-4 production, but not in IL-2 production (2, 3, 6, 7, 18, 19). Our detection of a defect in IL-2 production which is selective for responses to recall antigens and to self-APC processed alloantigens (ALLONW) but not to stronger stimuli such as ALLO or PHA further supports a deficiency in HLA self-restricted Th that is dependent on functional APC responses. The failure of other studies to detect IL-2 defects (6–8) is likely due to the potent stimuli used to activate T cells in those studies. Thus, to appropriately assess functional T cell development that is relevant for infection, the immunologic parameters tested should include responses to recall antigens or APC-depleted, allogeneic adult APC, none of which provide additional costimulatory factors. Furthermore, stimulation of infant PBL with APC-depleted, allogeneic adult APC provides a test of the infants' APC function without the complication of requiring primed T cells for responses to recall antigens.

This study demonstrates the importance of using a panel of immune stimuli to evaluate immune maturation that can distinguish between defects in helper, antigen presenting, and memory function. Based on our findings, it is unlikely that PBL from infants will be able to consistently respond by in vitro Th tests to recall antigens until ~ 12 mo of age for antigens such as TET, to which the infants have been immunized. There may be even a longer delay in Th function to recall

antigens for which infants have not been intentionally immunized such as FLU. There is an alternative possibility for infants exposed to antigens in utero. It is possible that T cells in utero (as well as after birth) are sufficiently mature to respond to recall antigens and to develop memory markers if the APC are mature enough to process and present antigen. Our findings that CBL after as short as 28 wk of gestation can respond to ALLONW (which does not depend on memory), and that this potential is absent in infants between 6 and 12 mo of age, raises the possibility that these putative maturational effects of maternal cytokines would permit efficient immunization for T cell immunity in utero or perinatally, but not necessarily during the first year of infancy.

It should be noted that, due to accrual restrictions, this report has been necessarily limited to the study of four infants between the ages of 6 and 12 mo, and to nine between 12 and 24 mo of age. Due to the small number of infants studied, it will be important that these findings be verified by other investigators.

It may be relevant that we have recently detected HIV-specific Th response in the CBL after delivery of ~ 40% of infants from HIV seropositive mothers (Clerici M., et al., manuscript submitted for publication). The findings of the present report may provide insights into perinatal T cell immunity that may eventually permit in utero immunization against infectious agents to which certain newborn infants would be at risk. Such an approach could be particularly useful in pregnant women infected with HIV.

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