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Alterations in helper-inducer and suppressor-inducer T-cell subsets in human neonatal blood

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SUMMARY

The distribution of T-helper cell subsets of human umbilical cord lymphocytes was compared with that of adult peripheral blood lymphocytes. Cytofluorometric analysis revealed a similar Leu 3/Leu 2 (helper/suppressor) ratio in neonates compared with adults. Total T-cell numbers were slightly decreased in neonatal blood. The Leu 3-positive-4B4-positive cell (helper-inducer) subset, however, was markedly reduced in neonatal blood, while the Leu 3-positive-2H4-positive cell (suppressor-inducer) subset was increased compared with that of normal adults. These findings may contribute to the poor help and enhanced suppression observed *in vitro* with neonatal T cells.

The increased susceptibility of human neonates to infection is multifactorial, but a relative state of immuno-incompetence appears to exist. One manifestation of this immuno-incompetence is a poor antibody response to antigenic challenge. The basis of this poor antibody response has not been completely elucidated and reflects defects at multiple levels.

Immunization of the human neonate induces low-level antibody responses that are initially restricted to the IgM isotype (Andersson *et al.*, 1981). Immaturity of both neonatal B and T lymphocytes is observed. Although B-cell precursors for IgG or IgA secretion are present in neonates in comparable frequency to the adult, the cells display an immature cell surface phenotype (Gathings, Lawton & Copper, 1977) and respond poorly to polyclonal B-cell activators *in vitro* (Andersson *et al.*, 1977; Gathings *et al.*, 1977; Griffiths-Chu *et al.*, 1984; Hayward, 1979; Hayward & Lawton, 1977; Morito, Bankhurst & Williams, 1979; Smith *et al.*, 1986; Tedder *et al.*, 1985a,b; Tosato *et al.*, 1980).

Recent studies indicate that within the human T-helper/inducer cell subset ($CD4^+$) there exists both phenotypic and functional heterogeneity. $CD4^+$ lymphocytes can be divided into two distinct subpopulations that bind one of two different monoclonal antibodies. Anti-4B4 (CDW29) defines a $CD4^+$ HI subset that provides help to B cells for antibody production (Morimoto *et al.*, 1985a). A reciprocal subset detected by anti-2H4 (CD45R) describes the suppressor/inducer (SI) T cell that induces suppressor and cytotoxic $CD8^+$ cells to generate effector cells (Morimoto *et al.*, 1985b). The 2H4 antigen has been shown to confer functional properties to the T cell (Morimoto *et al.*, 1986). The HI subset is also defined by the UCHL1 antibody (Smith *et al.*, 1986) while the SI subset corresponds to the HB10 and HB11 antibodies (Tedder *et al.*, 1985a,b). The two subsets show differences in proliferation to various stimuli. The 4B4 subset preferentially proliferates after stimulation with soluble antigens while the 2H4 subset proliferates in an autologous mixed lymphocyte reaction and after stimulation with concanavalin A (Morimoto *et al.*, 1985b, 1986). Both subpopulations proliferate in an allogeneic mixed lymphocyte reaction and after stimulation with phytohaemagglutinin.

In the present study we have quantified the T-helper cell subsets in neonatal blood and compared this distribution to that of the adult. This report describes differences in the numbers of HI (4B4) and SI (2H4) T cells between the two groups.

Umbilical cord blood was obtained from normal full-term vaginal or caesarean section deliveries. Adult peripheral blood mononuclear cells were obtained by venipuncture of healthy volunteers. Nucleated red blood cells were removed from neonatal blood samples by addition of a 1/7 dilution of 6·5% dextran sulphate solution (MW 252,000) followed by sedimentation at 37° for 30 min. Mononuclear cells were isolated by density gradient centrifugation over Ficoll–Hypaque. Residual red blood cells were lysed with a 1·0 M ammonium chloride solution.

Phycoerythrin-conjugated 4B4 and 2H4 antibodies were obtained from Coulter Immunology (Hialeah, FL). Phycoerythrin-conjugated Leu 2 and fluorescein-labelled Leu 4 and Leu 3 antibodies were obtained from Becton-Dickinson (Mountain View, CA). Leu 2a defines CD8-positive cells, while Leu 4 defines CD3 pan T cells. The Leu 3a and b antibody defines CD4-positive cells.

Cells, 10^6, were incubated for 30 min at 4° with the

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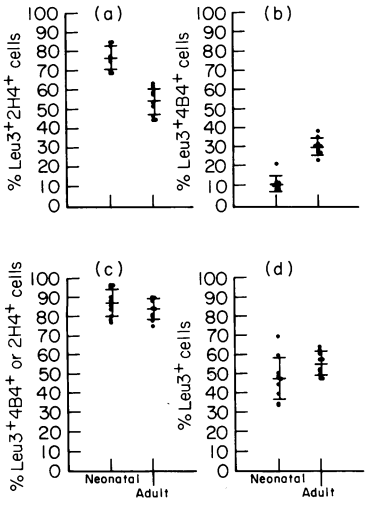

Figure 1. (a) Percentage of Leu3$^+$2H4$^+$ T cells in neonatal and adult blood. (b) Percentage of Leu3$^+$ and 4B4$^+$ T cells in neonatal and adult blood. (c) Percentage of T cells which are Leu3$^+$4B4$^+$ or Leu3$^+$2H4$^+$ in neonatal and adult blood. (d) Percentage of Leu 3$^+$ T cells in neonatal and adult blood. Percentages are shown as mean $\pm$ 2 SD.

appropriate monoclonal antibodies. Cells were then washed twice to remove unbound antibody.

Dual colour flow cytometric analyses were performed on a Coulter Electronics EPICS-C model cell sorter (Hialeah, FL) utilizing a 488 nm single beam argon laser line for fluorescence excitation. Green and red fluorescence were collected via a 525 nm band pass and 575 nm band pass filter, respectively. After appropriate gating on forward angle and 90 degree light scatter to identify the lymphoid component, at least 20,000 cells/sample were analysed. Corresponding negative controls for each stain were employed to determine the ranges of positive fluorescence. Three dimensional rotating data displays were utilized to confirm the placement of cursors separating fluorescent subpopulations on two parameter histograms.

Umbilical cord blood mononuclear cells that were isolated by Ficoll–Hypaque separation alone yielded approximately 60% nucleated red blood cells by differential staining. Cord blood samples treated with dextran sedimentation and lysis with ammonium chloride contained 1% nucleated red blood cells. The techniques employed to eliminate red blood cell precursors greatly aided flow cytometric gating steps, providing a much cleaner display and quantification of neonatal lymphoid cells. Dextran sedimentation did not alter the distribution of 2H4$^+$ or 4B4$^+$ cells in adult or cord blood samples (the authors' unpublished results).

The percentage of lymphoid cells comprising total T cells and a helper T-cell subset, as enumerated by monoclonal antibodies to Leu 4 and Leu 3, respectively, were 78% (62–91%) and 55% (47–64%) in the adult blood, and 66% (54–82%) and 46% (33–69%) in cord blood (Fig. 1d). Three dimensional profiles of stained and analysed cells demonstrate the separate subpopulations and the differences between neonatal and adult lymphocytes. The Leu 3/Leu 2 ratio in adults was 3·0 (1·7–4·4) while in the cord was 2·8 (1·7–5·3). In a total of 10 adults, the relative percentage of Leu3$^+$4B4$^+$ cells was 30% (23–38%) and of Leu3$^+$2H4$^+$ cells was 54% (45–62%). In contrast, in cord blood 10% (7–21%) Leu3$^+$4B4$^+$ cells and 76% (69–85%) Leu3$^+$2H4$^+$ cells were detected, which were both significantly different ($P < 0{\cdot}01$) (Fig. 1). The total percentage of Leu3$^+$ cells staining with antibody to either 2H4 or 4B4 was not different for cord and adult lymphocytes (Fig. 1c); these appear to be distinct subpopulations of the T-helper component. In addition, the peak intensity of staining of Leu3$^+$2H4$^+$ cells was not different in the cord and adult samples. However, this subset exhibited a greater fluorescence intensity than the Leu3$^+$4B4$^+$ component in both types of specimens.

Previous investigators have suggested the presence of immature T cells in human neonatal blood as a possible explanation for the inability of neonates to respond to certain antigenic challenges. In the current study the percentage of lymphoid cells representing the total T cells were slightly less in the newborn but the percentage CD4$^+$ and CD8$^+$ cells were similar in number in neonatal and adult blood. Human neonatal blood, however, showed a significantly decreased number of T HI cells and increased numbers of T SI cells. The finding of an increased number of T SI in neonatal lymphocytes is consistent with those of Tedder *et al.* (1985a) using the HB10 and HB11 antibodies (CD45R). An increase in the number of Leu3$^+$ cells that failed to bind either anti-2H4 or anti-4B4 antibody was not apparent in the neonatal lymphocytes. Whether there was an increase in the number of neonatal cells binding both antibodies could not be determined with the reagents used, but the sum of the individual subsets did not exceed 100% and was not greater than in adult blood.

Adult CD4$^+$ peripheral blood lymphocytes are reported to comprise of 40% 2H4$^+$4B4$^-$, 40% 2H4$^-$4B4$^+$, 10% 2H4$^+$4B4$^+$ and 15–30% 2H4$^-$4B4$^-$ (Morimoto *et al.*, 1985a,b). The developmental pathway of the four separate CD4$^+$ subsets defined by these markers has not been established. However, it is of interest that anti-2H4 antibody, in contrast to anti-4B4 antibody, fails to react with human thymocytes and does not bind to a limited number of immature human T-cell lines studied (Morimoto *et al.*, 1985a,b). The findings in this study demonstrating decreased number of 4B4$^+$ cells and increased numbers of 2H4$^+$ cells in the neonate in comparison to the adult is not easily reconciled with these findings. This present study investigated only blood lymphocytes. The disproportionate increase of 2H4$^+$ and decrease of 4B4$^+$ T4 cells may mean that cells that were successful in maturation and egress from the thymus may have expressed 2H4, which appears to be a functional antigen, on their surface.

Either a decrease in 4B4$^+$ or an increase in 2H4$^+$ T4 cells could explain the poor response of human newborns to antigenic challenge. Functional studies, however, will be required to elucidate the exact contribution of each to the lack of T-cell help and increased suppression observed *in vitro* with neonatal cells. Identification of alterations in number and function of T-cell subsets in neonates may help in developing new approaches to alter the newborn's immune response, which could prove important in prevention of the life-threatening infections that occur at that age.

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