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LYMPHOCYTE SUBPOPULATIONS IN THE BLOOD OF NEWBORN INFANTS

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SUMMARY

Assays of lymphocyte subpopulations and function have been applied to cells from the cord blood of twenty-four infants. The results are compared with those obtained in healthy adults. T cells, assayed by spontaneous rosette formation with sheep red blood cells (E rosettes) were present in lower proportion in cord (53%) than in adult blood (65%). There was a higher proportion of lymphocytes bearing stainable immunoglobulin in cord (32%) than in adult blood (22%). From the blood lymphocyte counts it was calculated that both T and B lymphocytes are present in greater numbers in the newborn infants' blood than in adults. Comparison of DNA synthesis showed that cord blood leucocytes had a higher spontaneous rate, but there were only minor differences in the lymphocyte mitotic response to phytohaemagglutinin (PHA). The response of cord blood lymphocytes was slightly lower to a submaximal stimulus and higher to a maximal stimulus. There was a correlation between the submaximal response and the proportion of E rosetting cells.

The most striking differences between infant and adult blood lymphocytes were in their cytotoxic activity against homologous target cells (Chang cells). Antibody-dependent cytotoxicity (K-cell activity) was readily detected using cord blood leucocytes, though it was lower than that of adult cells. PHA-induced cytotoxicity was very low in all cord blood samples, and in many cases was almost unmeasurable. This dissociation between the two types of cytotoxic activity is consistent with other evidence that they may be mediated by different cell types.

The assays were also applied to blood samples taken from five mothers of tested infants immediately after delivery. While some differences from normal adults were found with the mothers' lymphocytes they did not mirror those of the cord blood samples. This suggests that the pattern found for cord blood lymphocytes is not due to maternal factors crossing the placenta.

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INTRODUCTION

A variety of assays have been developed by which human lymphocyte subpopulations or their functions can be quantitated *in vitro*. Such tests are being increasingly applied to the investigation of disease states and to the measurement of the effects of immunosuppressive therapy. There is, however, little published data concerning physiological variations in lymphocyte subpopulations which may occur in relation to age. At one extreme, that of birth, it is easy to predict that there are differences from adults, since the blood lymphocyte count in newborn infants is normally about twice that of adults. It might also be anticipated that some differences would reflect the relative immaturity and 'virgin' state of the immunological system at birth. While there are a number of reports of the responses of cord blood lymphocytes to mitogens (Ayoub & Kasakura, 1971; Carr, Stiles & Fudenberg, 1972; Jones, 1969; Leikin, Mochir-Fatemi & Park, 1968; Pentycross, 1969) and of the presence among them of cells staining for surface immunoglobulin (Fröland & Natvig, 1972; Papamichail, Brown & Holborow, 1971), there have been no systematic quantitative studies of blood lymphocyte subpopulations in the newborn. We here report the results of such a study.

Apart from the blood lymphocyte count, the assays we have used are: spontaneous rosette formation with sheep red blood cells (E rosettes); immunofluorescent staining for surface immunoglobulin; mitotic response to phytohaemagglutinin (PHA); assays of both antibody-dependent and PHA-induced cytotoxicity against homologous target cells. The spontaneous formation of E rosettes is believed to be a marker for human T lymphocytes (Jondal, Holm & Wigzell, 1972), while the presence of stainable surface immunoglobulin is a characteristic of B cells. The mitotic response to soluble PHA is regarded as a property predominantly of T cells, although there is evidence that a proportion of B cells may respond in some circumstances (Phillips & Roitt, 1973; Phillips & Weisrose, 1974). The antibody-dependent cytotoxic lymphoid cell (MacLennan, 1972; Perlmann, Perlmann & Wigzell, 1972) is not thymus-dependent nor is it a conventional B lymphocyte. It has come to be regarded as a third lymphoid subpopulation, and has recently been termed the K cell (Editorial comment, *Nature: New Biology*, 1973). The nature of the cell whose cytotoxic activity can be induced by PHA is not clearly defined. The fact that this cytotoxicity is not abolished by removal of cells bearing surface immunoglobulin or Fc receptors (Perlmann *et al.*, 1972) suggests that it may be a T cell. There is additional evidence that antibody-dependent and PHA-induced cytotoxicity are mediated by different cell types (Holm *et al.*, 1974) and the results presented here are consistent with that evidence.

MATERIALS AND METHODS

Blood samples

Blood was collected from the umbilical cords of twenty-four infants at birth. Eighteen were healthy infants born by full-term normal delivery, one was a full-term infant delivered by Caesarian section, and the remaining five were pre-term (28–36 weeks gestation) but otherwise normal. In each case 8–12 ml of blood was defibrinated with glass beads, and an EDTA sample was taken for a white cell count and differential. Lymphocyte assays were performed on all samples within 24 hr of collection, and in most cases within 12 hr.

With each batch of cord bloods tested, a 'control' sample of venous blood taken from a healthy adult member of the laboratory or hospital staff was treated in the same manner.

As a further control, in case any differences detected in the cord blood lymphocytes might be due to maternal factors crossing the placenta, blood samples were also taken from five mothers immediately after delivery, and were tested at the same time as those of their infants.

Leucocyte harvesting

A crude preparation of leucocytes was obtained from the defibrinated blood by a modification of the gelatine sedimentation technique of Coulson & Chalmers (1964; Campbell *et al.*, 1974). The cells were spun down, washed in minimal essential medium (MEM), and resuspended in RPMI 1640 supplemented with 10% foetal bovine serum (FBS) (Biocult, batch 000239), glutamine, non-essential amino acids and antibiotics. The mononuclear cells were counted, and these cells were used for the assays of mitotic response to PHA and cytotoxic activity.

For rosetting and staining for surface immunoglobulin, lymphocytes were further purified by centrifugation (200 *g*, 40 min) on Ficoll–Triosil (specific gravity 1·085–1·088). The cells from the interface were washed twice in phosphate-buffered saline (PBS), pH 7·2.

Spontaneous rosettes with sheep red blood cells (E rosettes)

An aliquot of each sample of washed, purified, mononuclear cells (generally 10^6–3×10^6 cells) was resuspended in 0·2 ml of heat-inactivated FBS (HI-FBS) in a round-bottomed plastic tube ($\frac{1}{2}'' \times 3''$). Sheep red blood cells (Wellcome, in Alsever's solution) were washed in PBS and made up to a 2% suspension in HI-FBS, from which 0·2 ml was added to the lymphocytes. The tubes were incubated at 37°C for 15 min, and spun for 5 min at 150 *g*. They were left overnight at 4°C, after which most of the supernatant serum was pipetted off, and the cells were resuspended by gently rocking the tubes by hand. The whole cell suspension was taken into a Pasteur pipette and placed onto a glass slide. The drop was then smeared lightly on the slide with a coverslip, and rapidly dried. The smears were stained (May–Grünwald–Giemsa) and examined by conventional light microscopy. On each preparation 200–300 lymphocytes were counted, and the percentage of cells forming rosettes (three or more adherent SRBC) was recorded. This technique results in excellent, and permanent, preservation of rosettes, and allows the easy elimination of the few contaminating granulocytes which may be present.

Staining for surface immunoglobulin

An immunofluorescent technique was used, having first fixed the lymphocytes. Approximately 1 ml of ice cold 4% paraformaldehyde in PBS was added to an aliquot of the Ficoll–Triosil purified cells (10^6–3×10^6 cells) in a glass tube (1 × 5 cm), and left for 30 min fixation. The cells were spun down, washed twice in PBS and resuspended in PBS with sodium azide. They were then left overnight at 4°C in order to leach out any traces of fixative. The following morning the cells were again centrifuged and suspended in PBS with azide and 10% FBS. In this state they could be stored at 4°C until it was convenient to stain and count them among a batch of samples. For staining the cells were spun down and the pellet resuspended in 0·1 ml of a 1:3 dilution of fluorescein-conjugated sheep anti-human immunoglobulin (polyvalent, Wellcome). After 30 min at room temperature, unbound conjugate was removed by washing. A drop of the cells, suspended in phosphate-buffered glycerol (pH 8·5)

was placed on a slide under a coverslip. The percentage of cells showing staining was estimated by dark-ground fluorescent microscopy from a count of 200–300 cells.

Mitotic response to PHA

The unpurified mononuclear cells were adjusted to a concentration of 3×10^5/ml in RPMI 1640 plus 10% FBS. Cultures containing 1 ml of cells were set up in triplicate. PHA (Wellcome, dried reagent, Lot K6888) was added to two concentrations ($2:10^3$; $1:10^2$), the lower of which is a submaximal stimulating dose. Tubes were also set up without added PHA. Cultures were for 72 hr at 37°C. DNA synthesis was estimated by incorporation of [^{125}I] 5-iodo-2′-deoxyuridine (^{125}IUDR) (Craig, Garrett & Jackson, 1969). A stock solution of ^{125}IUDR (Radiochemical Centre, Amersham) was made up with added cold deoxyuridine to a concentration of 0·01 mg/ml and an activity of 20 μCi/ml. From this solution 0·1 ml (2 μCi) was added to each tube 3 hr before termination of culture. At the end of the culture, red cells were lysed by washing with 2% acetic acid in normal saline, and the residue was washed and spun down sequentially in normal saline, trichloracetic acid (twice), and methcol. This extensive washing was found necessary in order to reduce to low levels the isotope activity not specifically bound to the TCA-precipitated material. The radioactivity of the residues was counted in a Wallac Decem automatic gamma counter, and the results calculated as counts per minute (c.p.m.) after correcting for decay of the isotope and variations in counter efficiency. The results for PHA-stimulated cultures are expressed as c.p.m. after subtraction of the counts recorded in unstimulated cultures of the same sample.

Cell-mediated cytotoxicity

The technique used was similar to that described previously (Campbell *et al.*, 1974). The leucocyte suspension was made up to 3×10^5 and 10^5 mononuclear cells per millilitre in MEM plus 10% FBS. Target cells were Chang human liver cells, labelled by incubation for 1 hr at 37°C with 100 μCi of [^{51}Cr]chromate (Radiochemical Centre, Amersham). After labelling, the cells were washed by suspending them in 25 ml of MEM, layering 1–2 ml of FBS at the conical base of the universal container, and centrifuging the cells through the layer of FBS. In this way all the excess isotope was easily removed by sucking off the supernatant above the pellet of labelled cells. The Chang cells were counted, and made up to 10^4 per millilitre in MEM plus 10% FBS. Cultures were set up in triplicate with 1 ml of the leucocyte suspensions and 1 ml of Chang cells. Tubes were also included with medium only added to Chang cells. Three separate sets of tubes were set up: (1) with no reagents other than leucocytes and Chang cells; (2) with added heat-inactivated rabbit anti-Chang antiserum at a concentration of $1:10^4$; (3) with PHA at a concentration of $3:10^3$. These concentrations of antiserum and PHA are those which have been found to induce maximal killing of Chang cells by mononuclear cells. In the absence of such effector cells neither the antibody nor the PHA induce any isotope release above that occurring spontaneously from Chang cells in medium only.

The tubes were incubated for 20 hr, after which the percentage release of ^{51}Cr into the cell-free supernatant was computed from counts of the radioactivity in the supernatant and in the residue. From this percentage release, 'specific cytotoxicity' was calculated by correcting for the baseline release from Chang cells with medium alone (generally 15–25%) and the maximum ^{51}Cr release which we have found to be reached if the number of effector cells is increased indefinitely (Campbell *et al.*, 1974; MacLennan, 1972). Specific cytotoxicity was

plotted against the $\log_{10}$ of the number of mononuclear cells in the cultures. The relative cytotoxic activity of each sample of cells was then measured as the $\log_{10}$ of the number of cells required to produce 50% specific cytotoxicity. This is denoted SC_{50}. Since it represents the number of cells required to produce a particular level of killing, a high SC_{50} indicates low cytotoxic activity.

Expression of results

The assays as described give results which are related to the proportions of each lymphocyte subpopulation (or functional activity) present in a test sample. Estimates of the absolute level of such lymphocytes per unit volume of blood are likely to be more informative. As in previous publications (Campbell *et al.*, 1973, 1974) we have taken into account the lymphocyte counts in the tested blood samples. Thus, from the lymphocyte counts and the percentage of cells forming E rosettes or staining for surface immunoglobulins, we have calculated the absolute number of such cells present in 1 ml of blood. From the DNA synthesis measured in unstimulated and PHA-stimulated cell cultures, the mitogenic capacity was calculated as [$\log_{10}$-(lymphocyte count per millilitre of blood × c.p.m.)]. The cytotoxic capacity (either antibody-dependent or PHA-induced) was calculated as [$\log_{10}$ (lymphocyte count per millilitre of blood) − SC_{50}], and represents the cytotoxic activity present in 1 ml of whole blood.

The results of all the lymphocyte assays are plotted and expressed logarithmically. In the statistical treatment of the data, logarithmic means and standard deviations have been calculated. In analysis, the results obtained for the cord blood samples have been compared with the healthy adult controls tested in the same experiments, and with the mean results for healthy adults which we have established in a large series of assays.

RESULTS

The results of all the assays are summarized in Table 1 and Fig. 1.

Lymphocyte counts (Fig. 2)

As anticipated, the mean lymphocyte count in cord blood was more than twice that of normal adults.

E rosettes (Fig. 3)

A lower proportion of cord blood lymphocytes (mean 53%) formed spontaneous rosettes with SRBC than was found for adults (65%). The adult control group in this assay was too small to establish a statistically significant difference, but the difference between cord blood lymphocytes and the overall adult mean is highly significant (Table 2). This low proportion of rosetting lymphocytes is, however, outweighed by the greater lymphocyte count in the cord blood samples, so that the mean number of these cells per millilitre of blood is considerably higher than in adults.

Cells staining for surface immunoglobulin (Fig. 4)

The mean proportion of cells staining for immunoglobulin was 32% for cord blood lymphocytes, significantly higher than the value obtained for the adult controls (18·5%)

TABLE 1. Result of all assays

Assay	Cord blood	Adult controls	Normal adult range*	Cord blood vs adult controls *P* value†	Cord blood vs normal adult range *P* value‡
Blood lymphocyte count/mm^3	3·637±0·145 (24) (4335)	3·308±0·174 (9) (2032)	3·305±0·125 (89) (2018)	<0·001	<0·001
E rosetting cells	1·726±0·084 (20)	1·811±0·080 (5)	1·810±0·061 (49)	n.s.§	<0·001
Percentage	(53·2%)	(64·7%)	(64·6%)	(<0·1>0·05)	
Number per millilitre of blood	6·377±0·172 (20) (2·38×10^6)	6·075±0·054 (5) (1·19×10^6)	6·124±0·126 (49) (1·33×10^6)	<0·001	<0·001
Cells staining for Ig	1·509±0·105 (24)	1·267±0·142 (9)	1·340±0·165 (59)	<0·001	<0·001
Percentage	(32·3%)	(18·5%)	(21·9%)		
Number per millilitre of blood	6·147±0·204 (24) (1·40×10^6)	5·575±0·269 (9) (0·38×10^6)	5·638±0·190 (58) (0·43×10^6)	<0·001	<0·001
DNA synthesis	2·787±0·366 (23)	2·522±0·274 (8)	—	<0·025	—
Unstimulated c.p.m.	(612)	(333)			
Mitogenic capacity	9·430±0·354 (23)	8·726±0·158 (8)	—	<0·005	—
[PHA] 2:10^3, c.p.m.	4·113±0·464 (23) (12970)	4·286±0·306 (8) (19320)	—	n.s.	—
Mitogenic capacity	10·756±0·489 (23)	10·609±0·406 (8)	—	n.s.	—
[PHA] 1:10^2, c.p.m.	4·707±0·339 (23) (50930)	4·458±0·289 (8) (28710)	—	n.s. (<0·1>0·05)	—
Mitogenic capacity	11·35±0·357 (23)	10·78±0·320 (8)	—	<0·005	—
Cytotoxic capacity	5·61±0·38 (24)	5·04±0·41 (9)	4·98±0·37 (90)	<0·005	<0·001
Antibody SC_{50}	1·02±0·43 (24)	1·26±0·44 (9)	1·32±0·38 (78)	n.s.	<0·005
PHA SC_{50}	>7·12 (24)	5·35±0·33 (9)	5·37±0·43 (63)	<0·001	<0·001
Cytotoxic capacity	<1·5 (24)	0·96±0·39 (9)	0·94±0·42 (62)	<0·001	<0·001

Each result is expressed as the log mean ± 1 s.d., followed by the number of individuals tested in parentheses. Where relevant, the arithmetic equivalent of the logarithmic mean is shown below in parentheses.

* Established in a large series of assays using lymphocytes from healthy adults.

† Obtained by Wilcoxon's sum of ranks test.

‡ Obtained by Student's *t*-test on means.

§ n.s. = Not significant.

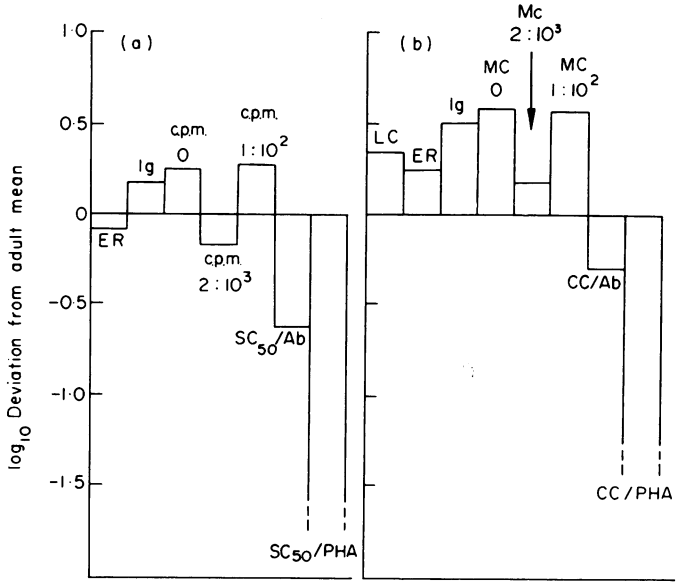

FIG. 1. Diagrammatic summary of results of all assays. (a) Proportional. (b) Absolute. For each assay the log normal adult mean has been taken as the baseline (0). Each block represents the logarithmic difference between the adult mean and the mean obtained with cord blood lymphocytes, for the assays indicated. LC = lymphocyte count. ER = E rosettes. Ig = cells staining for surface immunoglobulin. c.p.m. = Counts per minute in unstimulated and PHA-stimulated cultures. MC = mitogenic capacity. CC = cytotoxic capacity. For SC_{50}/PHA and CC/PHA no precise mean can be indicated, since in many cases this cytotoxic activity was too low to be quantitated.

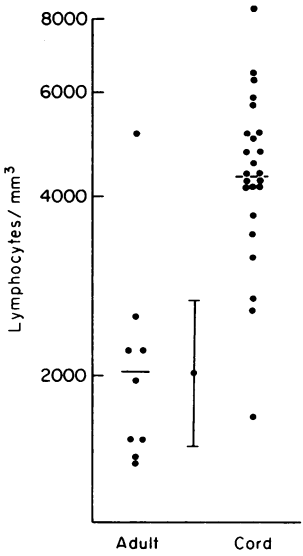

FIG. 2. Blood lymphocyte counts. The results (and mean) are shown for cord blood samples and adult controls. The central point and bar indicate the mean ± 1 s.d. for healthy adults, established in a large series of assays in this laboratory.

and the adult mean established in our laboratory (22%). These results indicate that the number of such lymphocytes is much higher in cord blood than in adults.

Mitotic response to PHA

Cord blood lymphocytes did not differ dramatically from adult controls in their mitotic response to PHA (Fig. 5). DNA synthesis in unstimulated cultures was greater for the cord cells. In the stimulated cultures, their mean response (after subtraction of the unstimulated c.p.m.) was slightly lower at the submaximal PHA dose, and higher at the maximal dose but these differences were not statistically significant. Taking into account the cord blood lym-

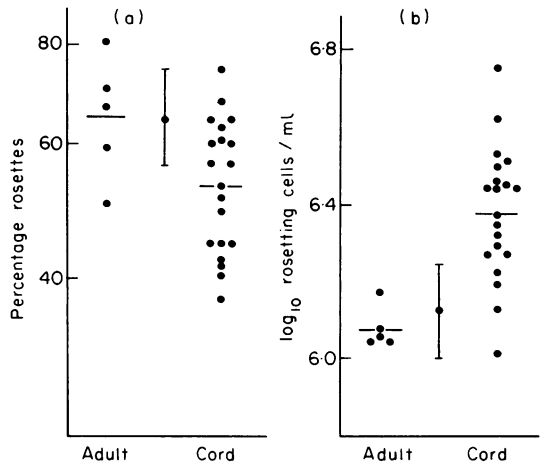

FIG. 3. E rosettes. The results (and mean) are shown for cord blood samples and adult controls. The central point and bar indicate the mean ± 1 s.d. for healthy adults. (a) Percentage of lymphocytes forming rosettes. (b) $\log_{10}$ Of the number of rosette-forming cells per millilitre of blood.

TABLE 2. Range of specific cytotoxicity* induced by 3×10^5 mononuclear cells against 10^4 Chang cells

Effector cells	Without antibody or PHA	With antibody	With PHA
Cord blood	2–14	16–82	7–21
Adult controls	6–22	44–98	38–71

* Specific cytotoxicity = corrected ^{51}Cr release (see Materials and Methods section).

phoctye counts, the mitogenic capacity of cord blood was significantly greater than that of the adults for unstimulated cells and for the higher dose of PHA (Fig. 6). (No 'normal adult ranges' are given for these assays of PHA response, as we have not yet accumulated a large series of results using precisely this assay technique.)

Cell-mediated cytotoxicity

The cytotoxic activity of cord blood lymphocytes was quite different from that of adults (Table 1, Fig. 7). Antibody-dependent cytotoxicity was readily detected, and although the mean activity, both proportional (SC_{50}) and absolute (cytotoxic capacity, Fig. 7), was lower than for normal adults, there was considerable overlap between the two groups.

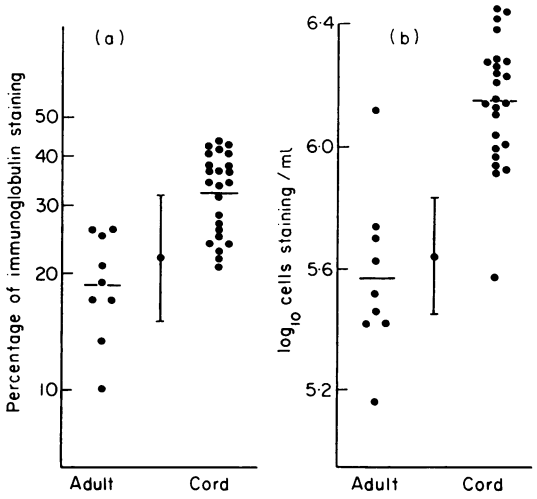

FIG. 4. Surface immunoglobulin. The results (and mean) are shown for cord blood samples and adult controls. The central point and bar indicate the mean ± 1 s.d. for healthy adults. (a) Percentage of lymphocytes showing membrane fluorescence when stained for immunoglobulin. (b) $\log_{10}$ Of the number of immunoglobulin-bearing cells per millilitre of blood.

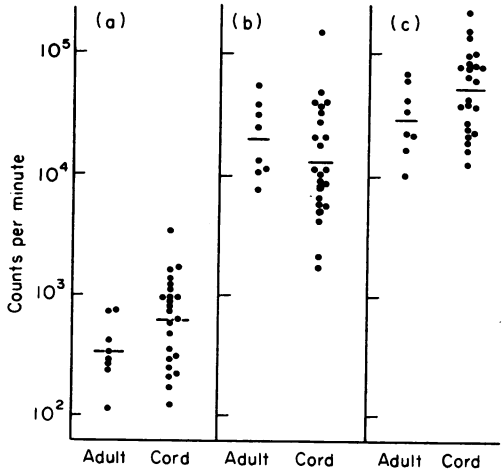

FIG. 5. DNA synthesis, measured by ^{125}IUDR uptake, by (a) unstimulated lymphocytes, and lymphocytes stimulated by PHA concentrations of (b) $2{:}10^3$ and (c) $1{:}10^2$. At the two concentrations of PHA, c.p.m. are shown after subtraction of the c.p.m. in unstimulated cultures. The results and mean are shown for cord blood samples and adult controls.

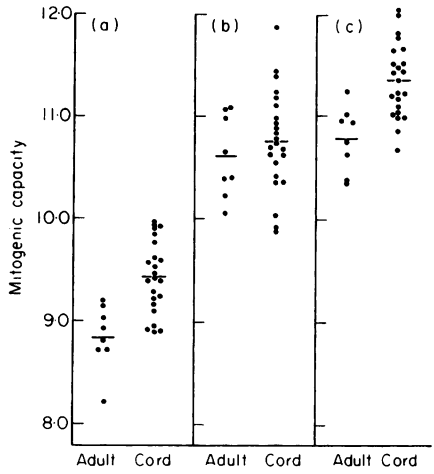

FIG. 6. Mitogenic capacity of blood, derived from c.p.m. and lymphocyte counts (see Materials and Methods section). (a) Unstimulated lymphocytes. (b) Lymphocytes stimulated with $2{:}10^3$ PHA. (c) Lymphocytes stimulated with $1{:}10^2$ PHA. The results and mean are shown for cord blood samples and adult controls.

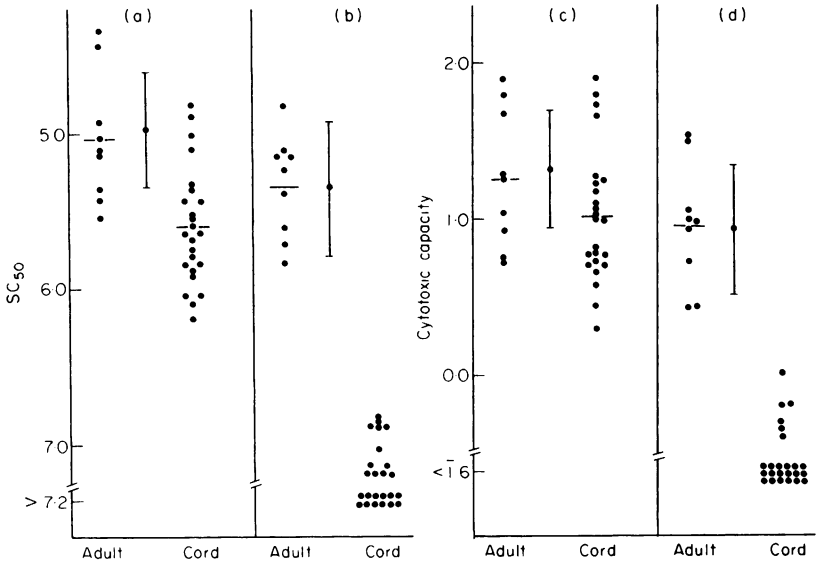

FIG. 7. (a) and (b) Cytotoxic activity of mononuclear cells against Chang cells, in the presence of (a) anti-Chang antiserum, and (b) PHA. (For explanation of SC_{50} see Materials and Methods section.) (c) and (d) Cytotoxic capacity of blood, indicating (c) the antibody-dependent and (d) PHA-induced cytotoxic activity present in 1 ml of blood. The results and mean are shown for cord blood samples and adult controls. The central point and bar indicate the mean $\pm$ 1 s.d. for healthy adults.

There was, however, a major difference in PHA-induced cytotoxicity. The addition of PHA induced only slightly more isotope release than was obtained with leucocytes alone. In many cases this cytotoxicity was so low as to be unmeasurable as SC_{50}. These lymphocytes, therefore, showed complete dissociation between the two types of cytotoxic activity.

Pre-term infants

Five of the infants tested were pre-term (28–36 weeks gestation). None of the assay results for these samples differed significantly from those obtained for the full-term babies. They have therefore been included, without being distinguished from the remainder of the results.

Maternal blood samples

Because of the small number tested, and for the sake of clarity, the assay results for the lymphocytes of the five mothers tested are not included in the figures and table. Their mean lymphocytes count was 2420/mm^3, slightly higher than that of normal adults. The mean percentage of lymphocytes forming E rosettes and staining for surface immunoglobulin were 72% and 25% respectively, both slightly greater than those of normal adults, but not showing a pattern similar to that of the infants' cells. Several of the mothers had low PHA responses. This was more clearly apparent with the submaximal dose (mean 2180 c.p.m.) than with the maximal (mean 19400 c.p.m.). Both the antibody-dependent and PHA-induced cytotoxic activities were lower than for normal adults (mean SC_{50} 5·62 with antibody and 5·80 with PHA). Both were, however, clearly present at a similar order of magnitude, and there was no dissociation between antibody-dependent and PHA-induced cytotoxicity, as seen for the cord bloods.

DISCUSSION

This study has revealed a number of differences between the lymphocytes of cord and adult blood (Fig. 1). The proportion of T cells, as assayed by E rosetting, is lower, and of B cells (surface immunoglobulin) higher, than in adults. However, because of the greater lymphocyte count in cord blood, the number of both these major lymphocyte subpopulations per millilitre of blood are greater than in adults. Our results indicate that there may be more than three times as many B cells in cord as in adult blood. This finding is in agreement with that of Fröland & Natvig (1972), who reported higher proportions of blood lymphocytes staining for IgG and IgM in newborn infants than in their mothers. It is interesting that there is such a high number of circulating B cells at a time when, unless there has been antigenic challenge *in utero*, little or no immunoglobulin production has begun (Good & Papermaster, 1964; Steihm and Fudenberg, 1966; Allansmith *et al.*, 1968) and few plasma cells have differentiated (Van Furth, Schuit and Hijmans, 1965). Perhaps these cells indicate a high rate of traffic from the human bursa-equivalent to the secondary lymphoid organs. Alternatively, they might represent a large pool of recirculating B cells, whose numbers will be progressively reduced to adult levels by a process of transfer from the recirculating compartment into the lymphoid organs and other tissues. Such a process might be conditioned by the continuing stimuli of contacts with extrinsic antigens which begin at birth.

The mitotic response of cord blood lymphocytes to PHA was not markedly different from that of adults. Their mean response was slightly lower at the submaximal, and higher at the

maximal stimulus, though the range of responses was wide. These undramatic differences are in accordance with the overall findings of other authors, who have variously reported lower (Ayoub & Kasakura, 1971; Jones, 1969), higher (Carr *et al.*, 1972) and similar (Leikin *et al.*, 1968; Pentycross, 1969) responses in cord compared with adult blood lymphocytes. In view of the lower proportion of T cells found among cord blood lymphocytes in this study, PHA responses might have been expected to be lower than for adults. It is clear from the numerically wide range of results observed when PHA response is measured as incorporation of radioactive nucleosides, compared with the relatively narrow range of variation in proportions of T cells as assessed by E rosettes, that there is not a simple proportional relationship between the number of T cells present and the PHA response observed. It is also apparent that qualitatively different results may be observed at different concentrations of PHA. It is our experience, in accord with that of others (Fitzgerald, 1971; Hosking, Fitzgerald and Simons, 1972) that the results of submaximal PHA stimulation show better discrimination between low and normal responses than those of maximal stimulation. It may be that in the culture conditions we have used, submaximal stimulation is a more selective T-cell assay. In the series of infant blood samples studied here, there is an overall correlation between the lower than adult mean proportion of E rosetting lymphocytes, and mean PHA response at the submaximal stimulus. We have analysed these data further, and have found a trend towards a linear relationship between PHA response and percentage of E rosetting cells (correlation coefficient = 0·493, $P<0{\cdot}05>0{\cdot}02$). No such correlation was found for the higher dose of PHA. These results suggest that under some conditions, and within a fairly homogeneous group of test samples, the PHA response can serve as a roughly quantitative assay of the proportion of T cells present in a particular sample of lymphocytes.

The most striking difference between the infant and adult lymphocytes was in their cytotoxic activity against homologous target cells. In the antibody-dependent system, K-cell cytotoxicity was readily detected among cord blood effector cells. Though it was proportionally lower (SC_{50}) for the infant cells, the mean cytotoxic capacity of the blood was quite close to that of adults, suggesting only a minor difference in the number or activity of circulating K cells. This substantial K-cell activity contrasts with the very low cytotoxicity induced by PHA.

These observations seem to conflict with two published reports. Carr, Lieber and Fudenberg (1970) detected considerable PHA-induced cytotoxicity by cord blood lymphocytes against chicken red blood cells, although in their paper no comparative data were given for adult effector cells. On the other hand, Rachelefsky *et al.* (1973) reported that the antibody-dependent cytotoxic activity of cord blood lymphocytes was extremely low. In their assay, the target cells were human lymphocytes, sensitized with HL-A antibodies, and the incubation time was 5 hr. Apart from the different target cells used in these two studies, it is difficult to explain the apparent qualitative differences from our results.

The K cell has been quite clearly characterized (MacLennan, 1972; Perlmann *et al.*, 1972) as a non-phagocytic, non-glass-adherent mononuclear cell, whose development is independent of the thymus. It does not carry detectable surface immunoglobulin (Greenberg *et al.*, 1973), but does have membrane receptors for the Fc portion of the IgG molecule. The nature of the cell type (or types) involved in PHA-induced cytotoxicity is less well defined. In the mouse it has been shown that the elimination of T cells diminishes but does not abolish this cytotoxic activity (Britton, Perlmann & Perlmann, 1973). Using human lymphocytes,

Perlmann *et al.* (1972) found that antibody-dependent cytotoxicity was almost completely abolished by passage of the cells through a Fc anti-immunoglobulin column, whereas PHA-induced cytotoxicity was little affected. Similar results have been obtained in our laboratory (Waller, Campbell and MacLennan, unpublished). The lymphocytes of human efferent lymph have almost no K-cell activity, but can show weak PHA-induced cytotoxicity (Holm *et al.*, 1974). These observations suggest that the two types of cytotoxicity can be mediated partly by different classes of cells. Though they do not clearly implicate a single cell type as responsible for PHA-induced cytotoxicity, T cells appear to be involved. In this respect it is interesting that our assays showed an absence of such cytotoxic activity in cord blood lymphocytes, despite the fact that many T cells were present, and in association with substantial proliferative response to PHA. It is difficult to interpret the significance of the lack of this killer function in the newborn. It might indicate either the absence or the functional immaturity of a particular subpopulation of cells, perhaps a subclass of T cells. Possibly it is a reflection of the immunologically 'virgin' state at birth, indicating the absence, not of a lymphocyte subpopulation, but of a functional capacity acquired after experience of antigen challenge.

Finally, the possibility was considered that some of the characteristics found for cord blood lymphocytes might be due to serum factors transmitted from maternal blood. There are a number of reports, for instance, of diminished PHA responses in late pregnancy, and of factors in maternal serum which can depress the response of lymphocytes from non-pregnant adults (Walker, Freeman and Harris, 1972; St. Hill, Finn & Denye, 1973). Although the cells in our tests were all washed, and no human serum was used in culture media, it is possible that such factors could have remained bound to the cell surfaces, modifying the results. We therefore tested blood samples from a small group of mothers at the same time as those of their babies. These maternal lymphocytes did give lower PHA responses than normal adults, and lower cytotoxic activity of both types. However, the overall pattern was quite close to that of normal adults, and quite different from that of the infants. We feel it is unlikely that the quantitative and qualitative differences between adult and cord blood lymphocytes are due to maternal serum factors.

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