

Analysis of Lymphocyte Proliferative Response Subpopulations in Very Low Birth Weight Infants and during the First 8 Weeks of Life

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ABSTRACT. Cell-mediated immunity is not well characterized in very low birth weight infants, and abnormalities may represent a significant vulnerability to infection. This report describes 165 serial studies in 58 infants between 700 and 1300 g birth weight during the first 8 wk of life. Two ml of blood were drawn at 2-wk intervals to measure T cell numbers and subsets and response to phytohemagglutinin (PHA). Overall, lymphocyte proliferation to PHA averaged 17,264 cpm, significantly less than the adult control (23,566 cpm). T cell numbers and subsets were CD3 62% (adult controls 75%), CD4 45% (49%), and CD8 18.6% (27%). Values at birth were lower as all parameters increased for at least the first 4 wk of life: PHA at birth was 15,464 cpm, CD3 48%, CD4 37%, and CD8 13%. Because of the lymphocytosis of premature infants, the absolute numbers of total T cells and subsets were within the normal adult range despite less than 50% of the mononuclear cells at birth being T cells. A study of five infants demonstrated an average of 52% B7+ cells at birth showing that the number of B cells at birth was increased approximately 10-fold over the control number in adults. Clinical correlation showed that the increases in both the % CD8 and the absolute number of CD8+ lymphocytes after birth were correlated with both the occurrence of sepsis and the number of red cell transfusions. In summary this study assessed lymphocyte subsets in a sizeable number of very low birth weight infants serially during the first 8 wk of life including lymphocyte function using isolated mononuclear cells. It demonstrated that premature infants are different from adults controls and full term newborns in: 1) having decreased lymphocyte proliferative response to PHA, 2) having lower % CD3 and CD8, and 3) having an increased number of B cells at birth. (*Pediatr Res* 23: 457-462, 1988)

Abbreviations

CMI, cell-mediated immunity
PHA, phytohemagglutinin
VLBWI, very low birth weight infants
RBC Tx, red blood cell transfusion

While there have been significant advances in neonatal medicine in the last 10 yr, as many as 20% of infants born in the weight range of 700 to 1300 g VLBWI do not survive (1). Many of these deaths can be directly or indirectly linked to infection. Despite the fact that abnormalities of CMI could represent a significant vulnerability to infection during early postnatal life, little information exists describing specific potentially important defects in CMI. Therefore, the investigation present herein was undertaken to characterize CMI in VLBWI where the effect on outcome might be most significant.

Previous studies of lymphocyte function and subsets in VLBWI are limited. Only two studies described proliferative responses in a small number of VLBWI (2, 3). No serial studies were performed and neither study used isolated mononuclear cells where intrinsic lymphocyte defects could be addressed. Three studies have described T cell numbers and subsets in VLBWI (4-6). However, B cell numbers were only investigated in one study (not with monoclonal antibodies) (4); studies were repeated in only one report (5) and the third study (6) described the percentage of T cell subsets as a percentage of isolated T cells preventing adequate comparison of their results with those of other studies.

In view of the lack of information characterizing CMI in VLBWI infants and the limited quantity of blood available, we chose to investigate basic parameters in order to begin to define levels of CMI in these premature neonates. Specifically these included lymphocyte function and subset analysis. We were able to use modified techniques (7, 9) to permit study of isolated mononuclear cells from a small quantity of blood and therefore to obtain data that could be directly correlated with existing information on term infants, older children, and adults. This report describes 165 serial studies in 58 premature infants of birth weight 700 to 1300 g including 126 determinations of the response to PHA and 137 determinations of T cell numbers and subsets. B cell numbers were assessed in five VLBWI.

METHODS

Patient selection. Fifty-eight of 78 babies of 700 to 1300 g birth weight who were in the New York Hospital Neonatal Intensive Care Nursery during the period of study were enrolled. Babies of mothers known to be at risk of being infected with HIV and babies with severe congenital anomalies would have been excluded from the study but did not occur in these 78 VLBWI. The protocol of study was approved by the Institutional Review Board of the New York Hospital and permission for testing was obtained from the parents of the babies. There were no obvious clinical differences evident between those infants entered in the

study and those not entered. Specifically there were no significant differences between the 20 infants not entered and the 58 who were enrolled in this study in regard to socioeconomic status, birth weight, rate of sepsis in the first 5 days of life and rate of intracranial hemorrhage as assessed by sonography. Since 70% of the VLBWI in The New York Hospital Neonatal Intensive Care Unit were born at other hospitals and transported in, some babies who were not enrolled represented the difficulty in obtaining consent under these circumstances. The plan of study involved performing the identical battery of tests (white blood cells with differential, response to PHA, and measurement of T cell numbers and subsets) serially at birth, 2, 4, 6, and 8 wk of age; no baby had blood drawn more than five times. Two ml of heparinized blood at room temperature were obtained at the time of each testing for a total of 10 ml during the entire study distributed over an 8-wk period. VLBWI less than 6 wk old when the study began were enrolled as were VLBWI less than 8 wk of age at the completion of the study. During the first month of study only lymphocyte response to PHA was assayed.

Flow cytometric analysis of T cell numbers and subsets. A total of 0.4 ml of the heparinized blood were removed for lymphocyte subset analysis by flow cytometry of whole blood with fluorescein isothiocyanate conjugated monoclonal antibodies CD3, CD4, and CD8 (obtained from Ortho Diagnostics, Raritan, NJ). A total of 0.1 ml was used per monoclonal antibody tested; the accuracy was verified by dilutional analysis (7). B7 was used in five VLBW to assess expression of B cell surface antigens and to quantitate the number of B cells (8).

Proliferative response to PHA. PHA response was carried out as previously described using a micromethod requiring only 50,000 cells/well (9). In brief, mononuclear cells were isolated by density gradient centrifugation, washed, and resuspended in RPMI 1640 with 25 mM HEPES buffer containing penicillin, streptomycin, 25 M glutamine, and 10% pooled normal human serum. Mononuclear cells were counted by light microscopy and plated at a density of 50,000 lymphocytes/well in triplicate. Three concentrations of PHA (75, 48, and 24 $\mu\text{g/ml}$ final concentration) were used to provide a sufficient range to insure that the maximum response was observed. Cultures were pulse labelled with 0.04 μl of [^3H]thymidine on day 3, harvested by automated Skaatron harvester on day 4 onto glass fiber filters, and counted in a scintillation counter. Data are given as median net maximum response. All studies were conducted in parallel with control blood obtained from hospital personnel as the internal standard.

Clinical correlation. Sepsis was defined as a positive blood culture in the appropriate clinical setting, *i.e.* hypotension, apnea, bradycardia, etc.

Table 1. T cell numbers and subsets: heparin vs EDTA and same day vs overnight

Control patient	Anticoagulant	Day of assay	CD3	CD4	CD8	T4/T8
1	EDTA	Same	65.9	36.4	27.9	1.3
1	Heparin	Same	62.7	34.7	27.9	1.2
1	EDTA	Next	65.1	39.6	26.7	1.5
1	Heparin	Next	63.3	37.4	28.0	1.3
2	EDTA	Same	68.6	46.5	24.3	1.9
2	Heparin	Same	72.2	44.1	24.1	1.8
2	EDTA	Next	69.6	47.7	24.9	1.9
2	Heparin	Next	70.7	47.6	24.6	1.9
3	EDTA	Same	79.9	40.9	33.4	1.2
3	Heparin	Same	81.7	42.8	32.2	1.3
3	EDTA	Next	82.3	48.4	32.2	1.5
3	Heparin	Next	81.1	43.1	33.2	1.3

Analysis of data. In the analysis of lymphocyte proliferative response to PHA and of the percentage and number of lymphocyte subsets, these parameters were described by means \pm SD. Differences between groups, *i.e.* VLBWI versus adult controls, were analyzed using the *t* test. The Spearman rank order correlation was used to investigate the relationships among different variables, *i.e.* sepsis and the percent and number of CD8+ cells. Analysis of variance was used to determine the probability that there had been a change in the PHA response, *e.g.* over the period from 0 to 8 wk postnatal age.

RESULTS

Experimental approach. Before beginning the study, tests were performed to ensure that the planned studies were feasible with 2 ml of blood and that only one anticoagulant would be required, because in general EDTA anticoagulated blood has been used for cell surface marker analysis. After 0.4 ml of whole blood was removed for surface marker analysis, the mean mononuclear cell recovery from the remaining 1.6 ml in VLBWI was 5.31×10^6 , much greater than that seen in adult normals and greatly in excess of the 0.60×10^6 cells required for the lymphocyte activation studies. The least recovery in 126 determinations of PHA was 0.63×10^6 cells. Table 1 demonstrates that there was no difference in the monoclonal antibody analysis when the blood was anticoagulated with heparin versus results obtained with EDTA. In contrast, studies of proliferative response to PHA using blood collected in EDTA revealed a clearly diminished response compared to simultaneous specimens collected in heparin (Table 2); and therefore heparin was used herein. In addition, Table 1 also demonstrates that there was no difference in surface marker expression as a result of routinely holding the specimens overnight before study.

Lymphocyte activation. The mean proliferative response to PHA in 126 determinations in 58 VLBWI was 17,264 cpm \pm 6539, substantially and significantly ($p < 0.01$) lower than a mean of 23,566 cpm \pm 5004 in 112 normal adult controls (Table 3; Fig. 1). Fully 40% of the determinations in the VLBWI were less than 16,191 cpm, which was the least response to PHA of any of the 112 adult normal controls.

Postnatal effects could be excluded as being responsible for the decreased responsiveness to PHA. As illustrated in Figure 1, the

Table 2. Lymphocyte proliferative response to PHA: heparin vs EDTA

Cell donor	Culture condition	Anticoagulant	
		Heparin	EDTA
1	Cells alone	35	30
	PHA 3*	18,732	9,866
	PHA 4	21,254	14,988
	PHA 5	18,076	14,351
2	Cells alone	25	45
	PHA 3	15,208	9,655
	PHA 4	22,919	14,657
	PHA 5	22,763	15,765
3	Cells alone	130	35
	PHA 3	12,387	7,460
	PHA 4	20,121	13,705
	PHA 5	21,069	14,697
4	Cells alone	25	30
	PHA 3	9,891	5,446
	PHA 4	14,607	9,615
	PHA 5	19,820	11,941

* Data presented as median net cpm for concentrations of PHA as follows: PHA 3 = 47 $\mu\text{g/ml}$; PHA 4 = 25 $\mu\text{g/ml}$; PHA 5 = 12 $\mu\text{g/ml}$.

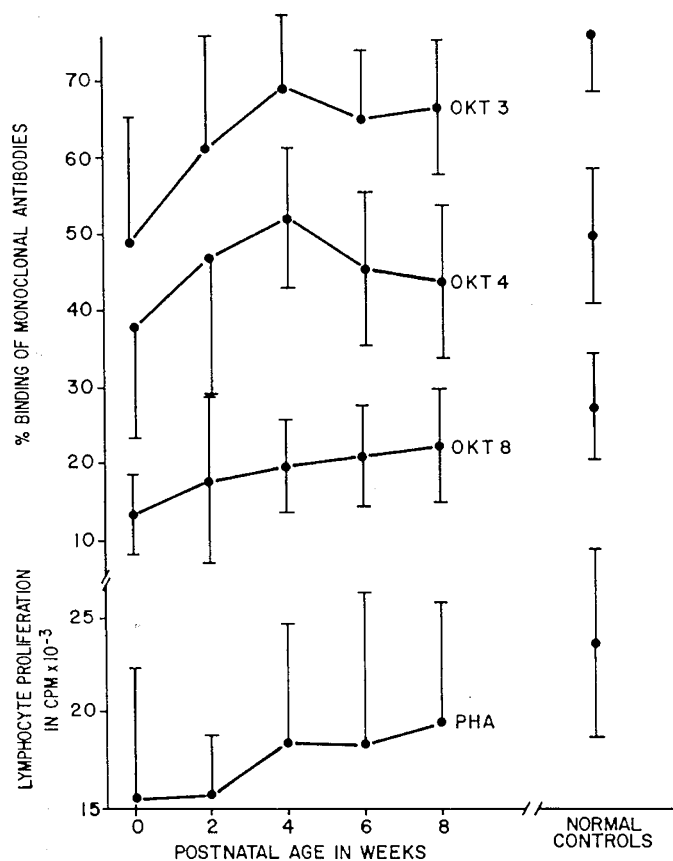


Fig. 1. The Y-axis is the level of CMI either in cpm (bottom, for PHA response) or in percent staining (top, for CD3, CD4, and CD8) expressed as the mean \pm 1 SD. The X-axis is postnatal age in two weekly intervals, the time interval at which the serial immune studies were performed. On the far right is the concurrent adult normal control data. Values for all parameters are low at birth and increase during at least the first 4 wk of life.

Table 3. CMI: all data*

Populations	T Lymphocytes				
	PHA	CD3	CD4	CD8	CD4/CD8
VLBWI	17264 (126) 6539	61.9 (135)	44.9 (136)	18.6 (137)	2.79 (136)
Adult controls (n = 112)	23566 5004	75.0 7.5	49.0 9.0	27.0 7.0	2.00 0.80

* PHA is presented as the mean, the (n), and the SD. The data for T lymphocytes are similarly presented. The n varied for the VLBWI from assay to assay and therefore is individually listed.

response to PHA tended to increase after birth ($p = 0.11$). In addition, subdividing the wk 0 data to compare specimens obtained on the first day of life to those from days 2, 3, and 4 (from babies born on Friday through Sunday) showed that day 1 proliferative responses tended to be less than those of days 2 to 4 (Table 4).

There was a wide range of proliferative responses to PHA evident both as patient to patient variability as well as inpatient changes when the same patients were restudied at different time points.

Lymphocyte subpopulation analysis: percentage of cell populations. Lymphocyte surface antigen expression in the VLBWI showed considerably and significantly decreased percentages of CD3 (62 versus 75%) and CD8 (18.6 versus 27%) positive cells

Table 4. CMI: initial values

	PHA	CD3	CD4	CD8	CD4/CD8
A: Initial values (wk 0)					
Mean	13304	44.9	35.8	14.0	3.07
SD (n)	4230(27)	18.2(29)	17.7(29)	10.7(29)	1.28(29)
B: Values on day 1 vs on days 2, 3, or 4					
Day 1					
Mean	13396	43.9	34.7	13.4	2.90
SD (n)	6014(17)	16.8(16)	13.2(17)	5.5(17)	1.50(17)
Days 2, 3, or 4					
Mean	17974	56.4	41.8	13.5	3.36
SD (n)	7363(14)	14.4(9)	14.6(10)	5.6(10)	0.83(10)

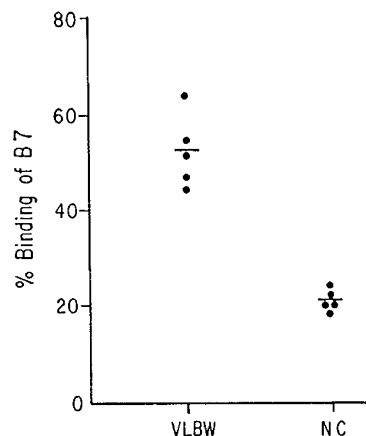


Fig. 2. The Y-axis represents the level of percent staining with B7 in five VLBWI and five concurrent adult normal controls with the horizontal lines expressing mean values. The X-axis indicates whether the results are from the VLBWI or normal controls (NC). The mean percent staining with B7 in the VLBWI was 52% compared to 21% in the NC indicating, when combined with the lymphocytosis of VLBWI, an at least 10-fold increase in the number of circulating B cells in VLBWI.

compared to normal adult controls; the level of CD4 positive cells (45 versus 49%) was not similarly reduced (Table 3). The mean %CD3 at wk 0 was only 48%: less than half the mononuclear cells at birth in VLBWI were T cells. Studies in five VLBWI demonstrated that the large percent of non-T cells appeared to be mainly B cells (Fig. 2) as evidenced by a mean of 52% binding with monoclonal antibody B7 at birth.

As illustrated in Figure 1, the percentages of cells expressing the surface markers defined by CD3, CD4, and CD8 increased after wk 0 (all $p < 0.003$). In addition, Table 4 shows that percent staining with all of CD3, CD4, and CD8 were lower for the day 1 specimens than for the day 2 to 4 samples confirming that postnatal events do not explain the decreased percent of T cells in the VLBWI.

The decreased percentage of CD8 cells combined with a normal percentage of CD4 cells yielded an increased mean CD4/CD8 ratio of 2.79. Only 3% of the specimens had CD4/CD8 ratios < 1.0 whereas 34% of the specimens had a CD4/CD8 ratio > 3.0 . Because the CD8+ cells continued to increase after 4 wk postnatal age, the CD4/CD8 ratio remained 3.0 between wk 0 and 4 and then decreased to 2.25 at wk 8 (Fig. 1).

Cell population numbers. The absolute number of lymphocytes at birth was elevated to approximately 5000/ μ l (Table 5) and this number remained stable for the first 8 wk of life in the VLBWI (see Fig. 3). The absolute number of B cells present at birth was dramatically increased to approximately tenfold that of normal adult levels (Table 4). Whereas the percentage of lymphocytes expressing surface antigens identified by CD3 and

Table 5. Absolute numbers of lymphocytes and lymphocyte subsets in VLBWI compared to normal adult controls

Absolute no. of:	VLBWI	Adult controls*
Lymphocytes	4500†	2314
OKT3+ mononuclear cells	2000	1687
OKT4+ mononuclear cells	1641	1091
OKT8+ mononuclear cells	620	564
B7+ mononuclear cells	2340	210

* Adult control data from Ballow *et al.* (5).

† Absolute cell counts/ μ l.

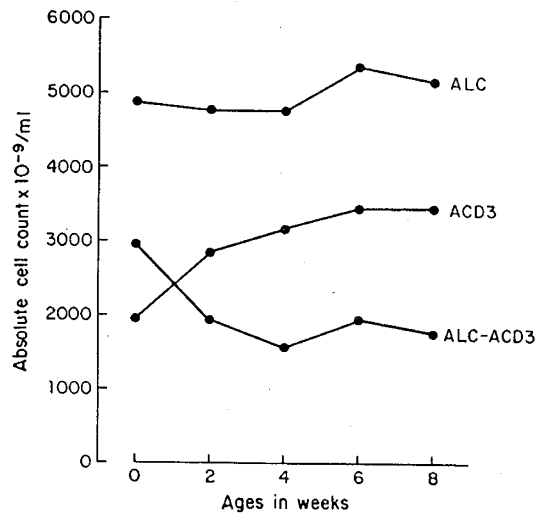


Fig. 3. The Y-axis represents the absolute number of cells $\times 10^{-9}/\mu$ l. The X-axis represents time in postnatal age at 2-wk intervals. ALC is absolute lymphocyte count and was derived from the Coulter counter white blood cell count multiplied by the percentage of lymphocytes. ACD3 stands for the absolute number of CD3+ cells derived by multiplying the ALC by the percentage of CD3+ cells. Inasmuch as the data suggests that ALC-ACD3 would approximately equal the absolute number of B cells (see "Results"), this parameter (ALC-ACD3) was included in the figure to represent the probable changes in the number of B cells in the 8 wk after birth in the VLBWI.

CD8 was significantly decreased at birth, the absolute numbers of T cells and their subsets were not decreased (Table 5; Fig. 4). There was a steady increase in the absolute number of CD8 staining cells during the first 8 wk of life in the VLBWI; CD3+ and CD4+ cells increased during only the first 4 wk of life and then declined slightly.

Proliferative responses to PHA did not correlate with the percent of mononuclear cells that were CD3, CD4, or CD8 positive or with the CD4/CD8 ratio, or the absolute numbers of lymphocytes, T cells, or T cell subsets and therefore was unlikely to be accounted for as a passive function of cell number or percentage.

Clinical correlation. Clinical correlations of CMI were investigated. Comparing levels of CMI in 26 survivors to five VLBWI who died, no significant differences were seen although the babies who died had lower mean values for all parameters of CMI (PHA, CD3+, CD4+, and CD8+). Birth weight and mode of delivery (vaginal *versus* cesarean section) were unrelated to parameters of CMI. Only three small for gestational age babies were included in this study; no substantial differences were seen in comparison to appropriate for gestational age babies.

The effects of clinical variables were also assessed by using the changes in the values of CMI between 4, 6, or 8 wk and birth (*i.e.* % CD8 at wk 8 minus % CD8 at wk 0. An increase in both

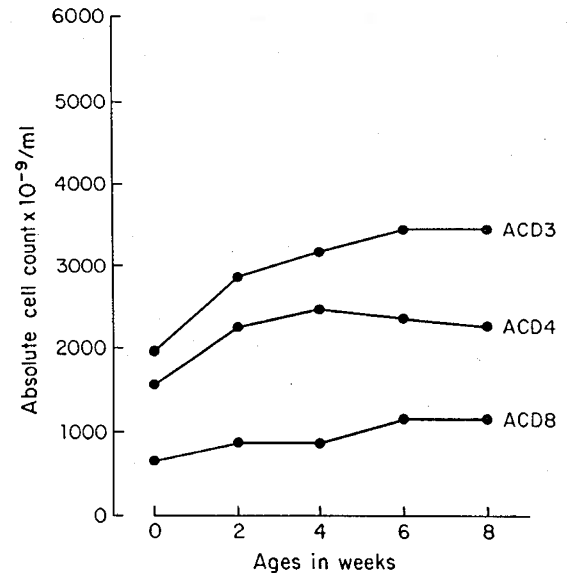


Fig. 4. The Y-axis represents the absolute number of cells $\times 10^{-3}/\mu$ l. The X-axis represents time in postnatal age at 2-wk intervals. ACD3, ACD4, and ACD8 represents the absolute number of CD3+, CD4+ and CD8+ cells, respectively. These numbers were derived by multiplying the absolute lymphocyte count (ALC) by the percentage of cells staining with the monoclonal antibody (CD3, CD4, or CD8) in question. As seen in figure 1 describing changes in the percent of total T cells and two T cell subsets, there is an increase immediately after birth in all of AT3, AT4, and AT8 but only AT8 was still increasing at 8 wk postnatal age.

the percent CD8 and in the absolute number of CD8+ lymphocytes correlated with both the number of red cell transfusions ($r = 0.465$ and 0.466 , $n = 22$, $p = 0.05$) and with the occurrence of sepsis ($r = 0.531$ and 0.482 , $n = 22$, $p \leq 0.05$).

DISCUSSION

This study demonstrates several important differences in parameters of CMI that distinguish VLBWI from both adult controls and (previous reports of) full-term neonates (10–12). These differences are: 1) decreased responsiveness to PHA; 2) lower percentage of cells expressing CD3 and CD8; and 3) higher percentage and number of cells expressing B7. Furthermore the data in Figures 1, 3, and 4 provide a comprehensive description of changes in lymphocyte function and subpopulations to VLBWI during the first 8 wk of life. Whereas the data include sick VLBWI and therefore cannot be used to define "normal" values for VLBWI, nevertheless they allow comparison of individual patients with the values outlined in the Figures 1, 3, and 4. These data suggest that the additional deficits demonstrated in VLBWI as compared to term infants could possibly underlie a component of the morbidity and mortality in VLBWI as has been previously shown in this population for delayed acquisition of humoral immunity (13–15) and for abnormalities of phagocytic cell function (16).

The results of the lymphocyte proliferation in response to PHA in VLBWI reported here and in both previous studies which describe lymphocyte proliferation (2, 3) demonstrate decreased lymphocyte responsiveness to stimulation with PHA. It is important to consider that all three studies stand in marked contrast to studies that have established that well full-term newborns have an at least equal to if not greater than normal adult response to PHA (10–12). However, both of the other studies of VLBWI reported a much greater percent decrease in PHA responsiveness than that reported in this study. These differences may be attributed to the use of whole blood in lymphocyte proliferation assays in the previous studies (2, 3) and to the use of cord blood (3).

Study of whole blood, in contrast to isolated mononuclear cells, makes comparisons between individuals difficult because such studies do not correct for the different numbers of lymphocytes in different individuals including the possible adverse effects of "crowding" and, in addition, serum factors may exert an influence on functional activity. Isolated mononuclear cells have not previously been investigated in VLBWI because 10 to 30 ml of blood, a volume that could only be recovered from the umbilical cord at delivery, were required to isolate the requisite number of cells. The lymphocytosis of VLBWI (5) and the ability to use microculture techniques (9) made the use of isolated mononuclear cells in assaying response to PHA possible.

The decreased responsiveness to PHA reported here may reflect an immaturity of the T cells in VLBWI, inasmuch as PHA stimulates primarily T cells (17). The mechanism could lie either in initial activation differences or in reduced secondary recruitment in the amplification phase of proliferation, perhaps related to soluble mediators such as IL-2. Studies in full-term newborns have implicated immaturity of neonatal macrophages in this process (18, 19).

A striking finding in this study was the high absolute number and percentage of non-T cells seen in the VLBWI. Whereas the overall percentage of CD3+ cells was 62%, at birth less than half of the mononuclear cells (48%) were CD3+. These results contrast with the findings of Ballow *et al.* (5) who reported 84% CD3+ cells at 1 wk of age. The discrepancy between their results and our own may lie in their technique of using buffy coats and washing it rather than direct assays of whole blood. The fact that there were 52% B7+ cells is consistent with the finding of 48% CD3+ cells and indicates that the non-T cells in VLBWI are mainly B cells. The percentage of monocytes in the differential white blood cell count was less than 8% in the five patients studied with B7 and cytofluorometry studies in an additional five patients using OKM1 also did not find these non-T cells to be monocytes (data not shown) suggesting that the "non-T" cells were indeed B cells. Thomas and Linch (4) also found an increased number of B cells in a small number of VLBWI. Term infants have been reported to have an increased percentage (and absolute number) of B cells, but not to the levels reported here (4). The greatly increased number of B cells in the peripheral blood may have been in transit to different areas of the reticuloendothelial system throughout the body at the time of (premature) birth. Whereas the VLBWI were not serially studied after birth for expression of B7, the significant increase in the absolute number of CD3+ cells presumably was concomitant with a decrease in circulating B cells as suggested in Figure 3.

The relative importance of the "percentage" that a given cell subpopulation is of the whole in comparison to the "absolute number" of cells of the same subpopulation present in a finite amount of blood is not known at this time. Certainly in VLBWI the T cell abnormalities are considerably more removed from adult normal when considered as percentages than when considered as absolute numbers (5; this study). The major persistent abnormalities in VLBWI are the large population of non-T cells which have been suggested herein to be B cells and the diminished T cell function evidenced by the reduced response to PHA.

The increased CD4/CD8 ratio found in the VLBWI was the result of a comparatively "normal" percentage of CD4+ cells but a decreased percentage of CD8+ cells; similar results have been previously reported (4, 5). Despite the decreased percentage of CD8+ cells, the absolute number of CD8+ cells was within the adult normal range. Term newborns have also been shown to have an increased CD4/CD8 ratio at birth although not to the same level as VLBWI (4, 20). The correlation between the increase in the percent and absolute number of CD8+ cells after birth with the number of RBC Tx and the occurrence of sepsis suggests that lack of environmental stimulation may contribute to the low percent of CD8+ cells at birth inasmuch as it implies that external stimuli may have long-lived effects on the percent and number of CD8+ cells.

Premature birth may not be solely responsible for the abnormalities in CMI in the weeks after birth, because "postnatal maturation" failed to result in the achievement of term neonate or adult levels of CMI by 8 wk postnatal age. In addition, only PHA and both percent and absolute number of CD8 were still increasing at that time. The one report available to clarify the course of further changes in CMI beyond 8 wk postnatal age in surviving VLBWI has indicated that the CD4/CD8 ratio remained persistently increased throughout the first year of life (5). Presumably the failure of CMI to achieve normal levels by 8 wk of age is also contributed to by postnatal events such as hyaline membrane disease and its associated complications and treatments. The apparent effects of RBC Tx and sepsis have already been alluded to and are consistent with other studies in older patients (20). Surprisingly, the birth weight (within the range 700–1300g) had no observable influence on the levels of CMI. Investigation of other parameters (survival, SGA, mode of delivery) provided less clear results, because of the small sample size. Inasmuch as postnatal events and interventions tend to be both dramatic and cluster together in the sickest VLBWI (*e.g.* ventilatory support, antibiotics, RBC Tx, vascular access lines, and sepsis), the influence of specific clinical events on CMI may be difficult to delineate without studying very large numbers of VLBWI infants.

In summary it is clear that T cell numbers, subsets, and function as well as B cell numbers are different from adult controls studied here and from previously reported term newborns. Furthermore these values did not normalize over 8 wk of follow-up. Whether the described abnormalities represent the absence of a maturation process that normally occurs *in utero* or rather pathologic events set in motion by the prematurity and/or postnatal events that occur at the premature birth is still not clear. This study implies that all of these factors may act in concert to produce the changes seen. Additional VLBWI need to be studied before the pathophysiology and consequences of the immunologic changes are better understood and any potential role of immunomodulation defined.

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ANNOUNCEMENT

Call for Abstracts

A medical and scientific symposium on the etiology, diagnosis, natural history, or treatment of Williams syndrome will be held on August 2, 1988, in Salt Lake City, UT. The deadline for abstracts is June 1, 1988. For additional information please contact: Frank Greenberg, M.D., Texas Children's Hospital, 6621 Fannin, Box 3-210, Houston, TX 77030, (713)791-4774.