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Lymphocyte subpopulations in peripheral blood of small-for-gestational age and appropriate-for-gestational age preterm neonates

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The lymphocyte subpopulations were investigated in peripheral blood of small-for-gestational age (SGA) and appropriate-for-gestational age (AGA) preterm newborns. In SGA newborns the number and percentage of T lymphocytes were reduced. Among the T lymphocytes, the number and percentage of T helper cells were significantly decreased. The cytotoxic/suppressor T cells were also reduced, but to a lesser extent.

A number of parameters of humoural and cellular immunity have been shown to be almost invariably impaired in protein energy malnutrition [7, 8, 9, 12, 22]. Numerical and functional deficiencies of T helper cells may be particularly important in the pathogenesis of the impaired immunity [11, 12].

Small-for-gestational age (SGA) infants exhibit several clinical features of a malnourished state including growth retardation, hypoglycaemia, hypothermia, increased susceptibility to infections, etc. [26, 28, 31, 33]. Like in older infants and in children with postnatally acquired malnutrition, the cell mediated immunity is compromised in SGA infants [6, 10, 13, 17, 27] and the impaired immune functions persist for several months to years [9, 10, 12, 13, 16].

Heretofore there have been no comprehensive analyses with specific monoclonal antibodies of the numerical and percentage distribution of pan T, helper and suppressor T cells in the peripheral blood of SGA preterm and appropriate-for-gestational age (AGA) preterm neonates. This paper presents our studies concerning the problem.

Materials and Methods

Sixteen low birthweight neonates were examined when they were one to two days old. Of the sixteen infants seven (four girls and three boys) were SGA and nine (five girls and four boys) were AGA. The diagnosis of intrauterine growth retardation was made on the basis of the birthweight below the 10th percentile for the corresponding gestational age of the standard fetal growth chart established for Hungarian infants [31]. Gestational age was estimated by maternal history of the last menstrual period and by neurodevelopmental examination of the neonates. The aetiology of intrauterine growth retardation was idiopathic placental insufficiency. The causes of preterm birth were undetermined.

None of the infants had evidence of infection, congenital malformation or metabolic disease.

From the one to two days old neonates peripheral venous blood was drawn for lymphocyte count and separation immediately after admission. Absolute lymphocyte count in G/L was determined in each sample using Hagedorn pipette and Buerker chamber.

Lymphocytes were separated from the heparinized venous blood by Ficoll—Uromiro gradient centrifugation [4]. Monocytes were removed from the aliquots used for surface marker analysis by adherence to a glass surface followed by carbonyl-iron treatment. Cell viability was determined using the trypan blue dye exclusive method.

The number of T lymphocytes was assayed by the E-rosette forming technique [24] and the B lymphocytes were counted by direct immunofluorescent method using FITC-labelled pig $F(ab)_2$ anti-human IgG (SEVAC).

Pan T, suppressor T and helper T cells were labelled with FITC-conjugated Leu-1$^+$ (Pan T), Leu-2$^+$ (suppressor T) and Leu-3$^+$ (helper T) specific monoclonal antibodies (obtained from Becton Dickinson Monoclonal Antibody Center, 490-B Lakeside Drive, Sunnyvale, CA 94086, USA). The different monoclonal antibodies were used by the method described in Becton Dickinson Procedures [3] and the different types of fluorescent cells were counted with an OPTON—S standard microscope equipped with fluorescence source (HBO 50 mercury lamp), a IVFL condensor and standard filters for FITC fluorescence.

Statistical analysis of the results was performed by Student's t test.

Results

Figure 1 shows the birthweight and gestational age of 16 infants, plotted on a standard percentile chart of fetal growth established for Hungarian newborns [31].

Table I presents the results for SGA and Table II those for AGA preterm babies. It can be seen that the absolute lymphocyte count in G/L was equal in the peripheral blood of the two investigated groups. The percentage of the SmIg bearing B lymphocytes was also comparable and there was no statistically significant difference between the two values ($0.20 > p > 0.10$). The proportion of E-rosette forming T, the Leu-1$^+$ (pan T) and Leu-3$^+$ (helper T) cells was significantly decreased in the peripheral blood of SGA babies, as compared to the AGA ones ($p < 0.05$ in all instances). There was no statistically significant difference between the values of Leu-2$^+$ (suppressor T) cells in the two groups ($0.50 > p > > 0.20$).

Discussion

The lymphocyte subpopulations in newborn infants showed great variations according to the method used and the results obtained [1, 15, 19, 21, 30]. Previous works concerning the lymphocyte subpopulations in newborns have been made on cells harvested from cord blood samples [1, 19, 20, 21]. The cord blood represents a limited part of fetal circulation exposed to contamination with maternal immunocompetent cells, especially expected to take place during birth [30]. Consequently we think that cord blood samples do not represent the real situation for newborn infants.

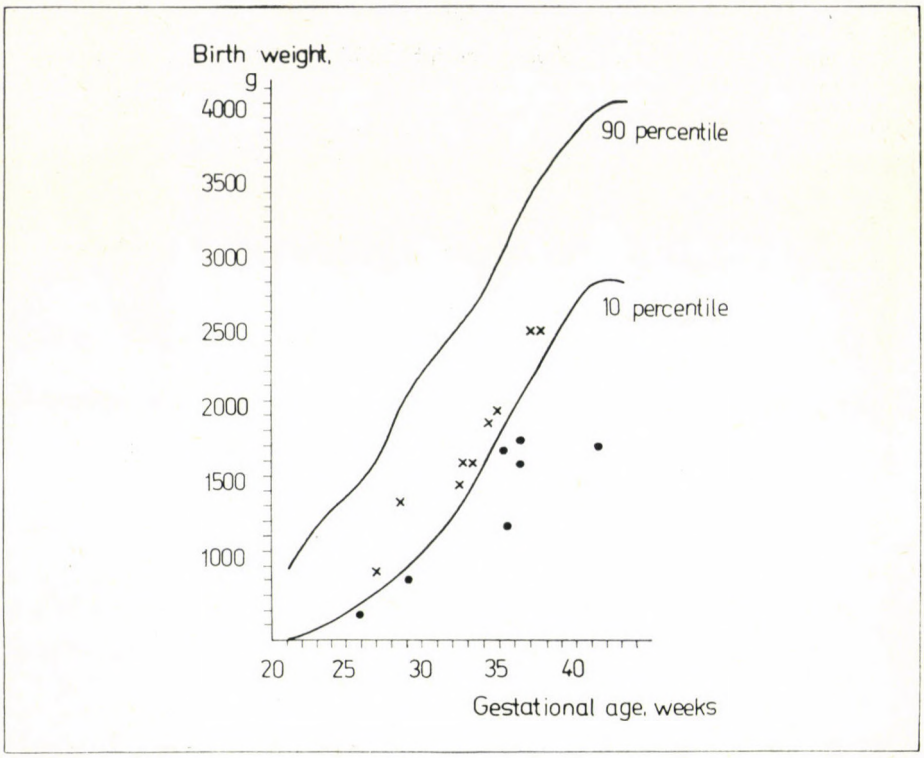

FIG. 1. Percentile standards for gestational age and birthweight for Hungarian newborns [31]

Hence we have preferred to examine peripheral venous blood lymphocytes.

The data obtained for the number and percentage of SmIg bearing B lymphocytes of our AGA preterm babies are in agreement with the results for full-term healthy infants [15, 20, 21, 30], although some authors

TABLE I

Lymphocyte subpopulations in the peripheral blood of SGA newborns

No.	Gestational age weeks	Birth weight g	Lympho-cytes G/L	SmIg+ cells	ER+ cells	Leu-1+ cells	Leu-2+ cells	Leu-3+ cells
				per cent				
1	41	1740	2.4	10	60	60	20	39
2	28	900	4.1	18	23	64	18	40
3	35	1180	3.2	11	64	66	29	37
4	36	1610	3.6	5	40	67	21	45
5	36	1770	2.7	14	41	68	31	36
6	34	1700	3.6	12	62	62	25	35
7	25	600	—	9	22	50	24	25
Average ± S.D.			3.2±0.6	11±4	44±17	62±6	24±5	36±6

TABLE II

Lymphocyte subpopulations in peripheral blood of AGA newborns

No.	Gestational age weeks	Birth weight g	Lymphocytes G/L	Smlg+	ER+	Leu-1+	Leu-2+	Leu-3+
				cells per cent				
1	28	1350	3.8	10	68	85	—	—
2	34	1930	2.1	12	70	75	33	42
3	32	1440	4.9	24	62	72	32	40
4	33	1600	2.5	10	68	71	31	39
5	37	2500	3.6	18	50	60	20	40
6	32	1600	3.8	26	40	64	23	40
7	26	880	3.0	17	60	66	18	40
8	33	1900	2.9	9	57	66	21	42
9	36	2500	—	9	73	74	28	43
Average ± S.D.			3.3±0.9	15±6	61±10	70± 7	26±6	41±1

reported on a higher percentage of B lymphocytes in full-term neonates [5, 18, 19, 27]. To compare the number and percentage of B lymphocytes is somewhat difficult, unless lymphocytes are induced to shed cytophilic Ig from their surface [21]. The disappearance of such passively absorbed Ig by incubation at 37°C has been documented for adults [25] as well as for newborn infants [21]. As a consequence of this treatment, the percentage of SmIg bearing B lymphocytes was comparatively low in our cases. For the time being, we are unable to compare our data for the number and percentage of B lymphocytes of AGA preterm infants, because to our knowledge no such results have been published.

We did not find any statistically significant difference between the relative frequency and absolute number of B lymphocytes of SGA and AGA preterm infants, but the frequency and number of B lymphocytes in the peripheral blood of SGA babies was lower. According to the observations of Moscatelli et al. [27] in the cases of full-term AGA and SGA infants, there was a significant decrease in the number of B lymphocytes in the peripheral blood of infants with fetal malnutrition.

The cord blood of healthy preterm and full-term newborns contains less E-rosette forming T lymphocytes than the blood of adults [5, 19, 21, 30]. Our data for AGA preterm newborns are in agreement with these results. We also observed that a considerable number of blood lymphocytes fails to form rosettes in spite of their reactivity with anti-T cell serum [2, 14]. They might be immature T lymphocytes, because during fetal ontogeny, T cells reactive with anti-T cell serum but failing to form rosettes are more numerous and probably appear earlier, than those bearing both markers [2, 14].

Among the SGA preterm infants both the E-rosette forming T cells

and the lymphocytes reactive with anti-T cell serum (Leu-1$^+$ serum) were decreased as compared to the data of AGA preterm newborns. The differences were significant statistically. Again, the decrease was much less with anti-T cell serum. Our observations agree with those who found a decreased number and percentage of E-rosette forming T cells in the blood of SGA infants [10, 13, 17, 27].

SGA infants exhibit several clinical features of malnutrition [26, 28, 31, 33]. Like in older infants and in children with malnutrition acquired postnatally [9, 11, 12] we found

(1) a decreased number and percentage of T lymphocytes;

(2) among the T lymphocytes a significant decrease in the number and percentage of T helper cells (cells reacting with Leu-3$^+$ serum);

(3) that the number of cytotoxic/suppressor T cells (cells reacting with Leu-2$^+$ serum) was also reduced, but to a lesser extent.

SGA infants have an increased susceptibility to infection and the impaired immunity persists for several months or even years [9, 10, 13, 16]. In malnutrition, the reduced ability to deal with many common infections may be due to inefficient antibody synthesis, which requires a normal number and functions of T helper cells [11, 12]. The reduced number and percentage of T helper cells in the blood of SGA infants is reminiscent of transient hypogamma-globulinaemia of infants [32]. In this disease, a delay of immunoglobulin production was found, which usually resolves spontaneously by 30 to 40 months of age [23]. The affected infants have a high incidence of infections and unexplained episodes of fever and bronchitis [29]. Siegel et al. [32] studied 17 patients with transient hypogammaglobulinaemia to define the immunologic defect responsible for the disorder. The number of circulating B cells was normal, as well as the ability of B cells to synthesize immunoglobulins when stimulated with Epstein—Barr virus, a direct B cell activator. The capacity of B cells to synthesize IgG in response to pokeweed mitogen, a T cell dependent B cell activator, was, however, depressed. A numeric deficiency in T cells was found. Patients who had recovered from this disorder, usually at 30 to 40 months of age, had a normal number of T helper cells at this time.

Development and maturation of the immune system in a full-term healthy newborn has largely been completed during intrauterine life. Adverse factors including nutritional deprivation during this critical phase may have a prolonged or even permanent effect on immunity. In the present study, impaired cellular immunity was demonstrated by the decreased number of T lymphocytes and by the decreased number of T helper cells in the cases of SGA infants. Further investigations are needed to determine how the functions of T helper cells could be influenced and whether the impairment might be reversible.

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