Int. J. Prenatal and Perinatal Psychology and Medicine Vol. 6 (1994) No. 1, 87–93

The Evaluation of Selected Parameters of the Umbilical Blood Lymphocyte Activity

L. Marianowski[1], *E. Skopinska-Rozewska*[2], *P. Kaminski*[1], *S. Majewski*[3], *M. Radomska*[2], *and M. Hibner*[1]

[1] From I Department of Obstetrics and Gynecology, Medical University of Warsaw. Head of Department: Professor L. Marianowski, M.D, Ph.D.
[2] From Department of Immunology, Institute of Tuberculosis and Lung Disease in Warsaw Head of Department: Professor E. Skopinska-Rozewska, M.D, Ph.D.
[3] From Department of Dermatology, Medical University of Warsaw. Head of Department: Professor M. Blaszczyk-Kostanecka, M.D, Ph.D.

Abstract

The angiogenic activity of mononuclear cells, activity of NK cells was examined. Subpopulations of T lymphocytes in the umbilical blood was examined using the teophilline test.

Blood for the study was drawn immediately after the delivery to the heparinized syringes by the puncture of the umbilical vein. Mononuclear leukocytes were isolated using the modified Boyums method. The activity of the NK cells was assessed using Cr^{51} labelled leukemia line K-562 as the target cells.

The LIA test was performed on the 6–8 week old inbreed BALB/c mice after the immunosuppression. The results were readd in the surgical microscope using the morphological criteria.

It was found that the mean number of the newly formed vessels in the LIA test was 12.85 ± 0.2 (SE) and the mean percentage of the natural cellular cytotoxicity was 7.05 ± 0.9 (SE).

Zusammenfassung

Die T Subpopulation von Lymphozyten, die Aktivität von NK Zellen und die angiogene Aktivität von mononiklearen Zellen im Blut von

Correspondence to: Professor L. Marianowski, M.D, Ph.D., I Department of Obstetrics and Gynecology, Medical University of Warsaw, Pl. Starynkiewicz 1/3, 02-015 Warsaw, Poland

52 gesunden Neugeborenen wurde untersucht. Die Durchschnittszahl von neu gebildeten Gefäßen im LIA Test war 12.85 ± 0.2 (SE) und der mittlere Prozentwert der natürlichen zellularen Zytotoxizität war 7.05 ± 0.9 (SE).

*

The development of mechanisms being responsible for immunocompetency of the child begins in the second month of in utero life. First lymphocytes appear in the fetal blood between 7th and 8th week of pregnancy[1]. It was assessed that 90 % of fetal T lymphocytes, between 15th and 20th week of pregnancy is capable of rosette forming with sheep RBC's. The activity of NK cells can be observed in the fetal liver in the 9th week of pregnancy and in the circulating blood in 28–33 week of pregnancy.[17]

At the term, newborn has well developed cellular response, and not fully developed humoral response, represented by physiologic dysimmunoglobulinemia. Additionally, nonspecific immunity which plays important role in the overall immunity are not yet matured and mature with the development and growth of the child. The efficiency of immunologic system increases with age and depends on the personal traits, contact with foreign antigens and environmental factors. The peak of immunologic competency is reached by the age of 18.

One of the most sensitive qualitative methods evaluating cellular activity is Lymphocyte Induced Angiogenesis LIA developed by Sidky and Auerbach[9]. There are the suggestions that angiogenic activity of mononuclear cells changes according to the age.[6,7]

Aim of the Study

The aim of the study was to evaluate certain parameters of cellular immunity: NK cells activity, angiogenic activity of mononuclear cells and lymphocyte T subpopulation in the umbilical blood of the healthy newborns.

Material and Method

The study was conducted on the 51 healthy newborns after normal pregnancy and delivery. Babies were delivered between 36th and 42th week of pregnancy and according to Apgar score were in good health.

Blood was drawn from the newborns directly after the delivery from the umbilical vein to the syringes with 1–2 drops of heparin. Mononuclear lymphocytes were isolated by modified Boyuma method[3] that is centrifugation of blood in the gradient of Ficoll and Uropolin. After 20 minutes of centrifugation 450 g the cells were washed twice in 0.9 % NaCl and brought to the concentration of 10×10^6 cells in ml of rosette environment (10 % inactivated and adsorbed human serum AB group in PBS). In teophilline test lymphocytes were incubated with 0.01M teophilline in the rosette environment 1.5 hour in 37 °C with the control (incubation in the environment). After the careful wash rosette E test was conducted. 0.1 ml. lymphocytes in the concentration 5×10^6/ml was added

to 0.1 ml 250 × 10^6/ml SRBC (sheep red blood cells). ARFC samples were conserved immediately, and complete rosettes (TRFC) were incubated in 4 °C for 18 hours.

The activity of NK cells was assessed using leukemia K-562 cells as target cells.[4] Target cells were cultured in the RPMI 1640 (Sigma) medium and labelled with ^{51}Cr by incubation with 100 μCi/ml $Na_2{}^{51}CrO_4$. Those cells (2 × 10^6) were then mixed with efector cells (1 × 100^6) and incubated for 4 hours in 37 °C. After the incubation the cells were centrifuged and supernatant radioactivity of liberated ^{51}Cr was measured. (gamma radiation counter, Amnes Miles).

Cytotoxic percentage was calculated from the formula:

$$\% \text{ of cytotoxicity} = \frac{\text{experimental liberation - spontaneous liberation}}{\text{maximal liberation - spontaneous liberation}} \times 100$$

Spontaneous liberation was observed with the target cells incubated with control medium, and maximal liberation with the cells incubated with Triton X-100. The essays were repeated three times.

LIA test was done on the 6–8 weeks old inbred mice BALB/c treated immunosuppressivley with the intraperitoneal injections of cyclophosphamde 200 mg/kg body mass, 4 hours before the test. Under anesthesia with chloral hydrate cells were injected intradermally (10^6/ml Parker medium according to the method described by Sidky and Auerbach with small modifications). In order to visualize the place of cells injection 0.01 % of tryphan blue was added. 72 hours after the injection, the recipients were killed and skin was cut apart from the deeper tissue. Test results were read in the surgical microscope with magnification × 32. All newly formed vessels were counted in the circle with the diameter equal to the half of the field of view. Counted vessels had small diameter, highly curved and numerous anastomoses.[10]

The results of function tests and lymphocyte subpopulation were compared to the results from healthy adults and children. Control groups were examined by E. Skopinska-Rozewska[11,12,13,14] and S. Majewski[4].

Results

The results of the study are presented below. Table I shows the results of the function tests compared to the results obtained from the healthy adults.

The mean natural cellular cytotoxicity in the examined group compared to the control group is significantly lower ($P < 0.001$). The mean number of newly formed vessels in the LIA test in the group of newborns is higher than in the group of adults ($P < 0.01$).

Table II shows the mean percentage of early, late, total teophilinoresistantant teophilinodependent subpopulations of T lymphocytes in the given age groups.

The mean percentage of teophillinoresistant active rosettes increases with age: 11.2, 37, 42.5, 48.9. It is lowest in newborns ($P < 0.01$). The percentage of the other fractions is also the lowest in the newborns but no other correlation can be seen.

Table I. The results of function tests (NK and LIA) in correlation to the results obtained from healthy adults.

	NEWBORNS	HEALTHY ADULTS	SIGNIFICANCE
CYTOTOXICITY % E/D 50/1	7.05 ± 0.9 * n=52	36,6 ± 2.5 * n=20 [a]	P<0.001
ANGIOGENESIS (the mean number of new vessels)	12.85 ± 0.2 * n=31	12.2 ± 0.09 * n=140 [b]	P<0.01

* mean value ± SE
[a] results by S. Majewski et al.
[b] results by E. Skopinska-Rozewska et al.

Table II. Teophilinodependent and teophillinoresistant T lymphocytes forming early (ARFC) and late (CRFC) rosettes with sheep RBC's in different age groups.

	TEOPHIOLLINO-RESISTANT RFC			TEOPHILLINO-DEPENDENT RFC		
AGE GROUP	ARFC	CRFC	TOTAL	ARFC	CRFC	TOTAL
NEWBORNS n=30	11.2 ± 1.4	16.8± 2.4	28.0± 2.7	2.1± 0.7	2.0± 0.7	4.1± 1.1
CHILDREN AGE 1-14 n=180	37± 2	8± 1	45± 2	9± 1	7± 1	16± 2
ADULTS AGE<50 n=46	42.5± 1.7	25.7± 1.4	67.2± 1.8	6.9± 0.9	3.2± 0.6	10.1± 1.2
ADULTS AGE >50 n=32	48.9± 2.5	11.9± 2.1	60.8± 2.7	7.7± 1.1	4.2± 0.8	11.9± 1.3

Statistically significant difference between the group of the newborns and other age groups is seen at $P < 0.01$. The results of the control groups were obtained by E. Skopinska-Rozewska et al.[1,52,13,14].

Figure 1 shows the correlation between TRFC and the angiogenic activity of mononuclear cells.

Figure 2 shows the correlation between TRFCres and the angiogenic activity of mononuclear cells.

Figure 3 shows the correlation between teophilinoresistant fraction of late rosettes and angiogenic activity of mononuclear cells from the umbilical blood.

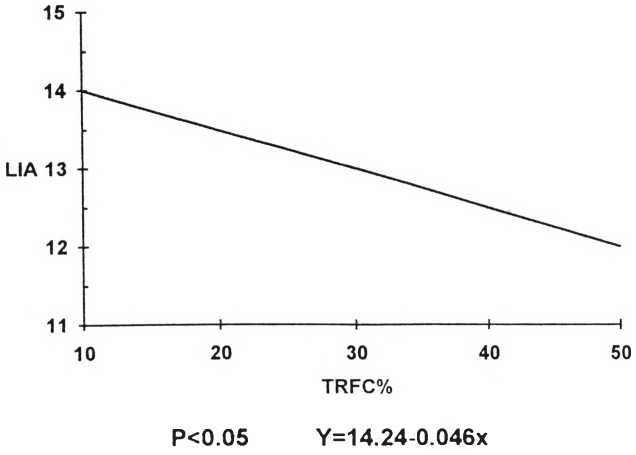

Fig. 1. Negative correlation between TRFC and LIA test with $P < 0.05$.

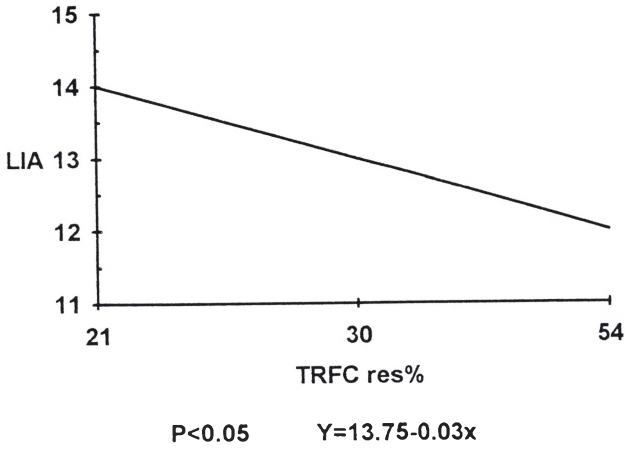

Fig. 2.

Discussion

Among the analyzed results in newborns the biggest deflection from normal is seen in the assays of natural cellular cytotoxicity. NK cells play an important role in anti-viral and anti-tumor immunity. They also have important regulatory functions. The activity of NK cells from the umbilical blood is lower than in adult blood and the activity of NK cells in the blood of the examined newborns is lower compared to the results presented by the other authors[15,16,17]. The study was conducted in the newborns of the healthy mothers after normal pregnancy. It can be concluded that the lowered NK cells activity may be physiological in the neonatal period and not dependent on the exogenic factors. Lowered NK cells activity

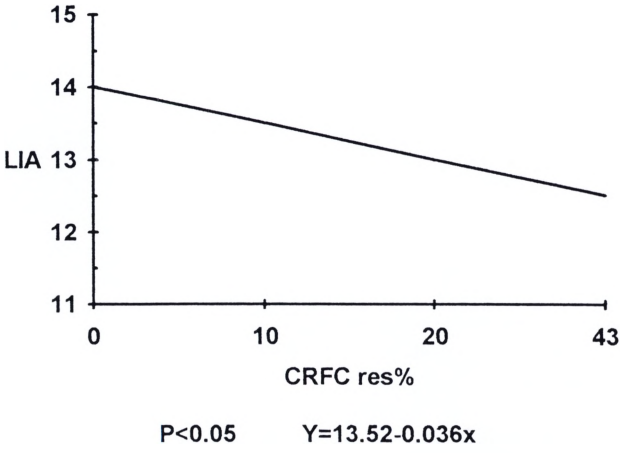

Fig. 3. The correlation between CRFCres and LIA is negative with $P < 0.05$.

may be due to diminished number of circulating lymphocytes or impairment of their function.

The study of angiogenic activity of mononuclear cells in the umbilical blood showed its increase compared to one seen in adult blood. This is surprising, because young organism should show high angiogenic activity. Physiologically angiogenesis occurs during growth and differentiation of the organism, it has the important role in organogenesis.

Normal production of angiogenic lymphokinins by newborn lymphocytes is not always followed by the normal secretion of other protein substances. Lowered interferon production has been reported[18,19]. On the other hand the production of interleukin-2 reaches normal levels in the neonatal period[8]. In the umbilical blood the higher number of non mature subpopulations of T lymphocytes is seen, it is possible than that there are other cells as macrophages and granulocytes responsible for the angiogenic activity.

The negative correlation between the total percentage of T lymphocytes and angiogenic activity of mononuclear cells was found. Further analysis showed that the cells that correlated negatively with LIA test are the teophilline negative fraction of complete rosettes, and in this group the subfraction of the cells of late rosettes having the receptors with the lowest affinity to the sheep RBC's. This observation is consistent with the studies by other authors[3,20].

Conclusions

Finding of distinct deviations among some parameters of the cellular immunity in the umbilical blood of the healthy newborns suggests differentiation of maturation mechanisms of this type of immunity in fetal life.

References

1. Gornicki, B., Debiec B. (1985). *Pediatria vol. I.* Warszawa, PZWL
2. Kaminski, M. et al. (1981). *Arch. Immunol. Ther. Exp.* **29**, 521
3. Kaminski, M. et al. (1981). *Clin. Exp. Immunol.* **46**, 327
4. Majewski, S. et al. (1987). *Br. J. Dermatol.* **116**, 1
5. Nair, M.P.N. et al.(1985). *Cell Immunol.* **94**, 159
6. Polakowski, I.J. (1986). *Praca doktorska.* Warszawa
7. Polakowski, I.J. et al. (1987). *Pol. Tyg. Lek.* **19**, 595
8. Roehm, N.W. et al. (1981). *Cell Immunol.* **63**, 272
9. Seki, N. et al. (1985). *J. Immunol.* **4**, 2351
10. Sidky, Y.A., Auerbach, R. (1975). *J. Exp. Med.* **141**, 1084
11. Skopinska-Rozewska, E. et al. (1988). *J. Invest. Dermatol.* **90**, 851
12. Skopinska-Rozewska, E. et al. (1988). *Folia Biol.* **34**, 58
13. Skopinska-Rozewska, E. et al. (1989). *Pol. Tyg. Lek.* **6**, 147
14. Skopinska-Rozewska, E. et al. (1991). *Arch. Immunol. Ther. Exp.*
15. Toder, V. et al. (1982). *Clin. Exp. Immunol.* **48**, 649
17. Uksiala, J. et al. (1983). *Clin. Exp. Immunol.* **1**, 153
18. Wakasugi, N., Virelizier, J.L. (1985). *J. Immunol.* **1**, 167
19. Wakasugi, N. et al. (1985). *J. Immunol.* **1**, 173
20. Zukowska, M. (1989). *Praca doktorska.* Warszawa