Count and Heterogeneity of Human Fetal Blood Lymphocytes in the Course of Fetal Development

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Blood specimens from 89 human embryos and fetuses were analyzed by immunological methods during gestation weeks 4-40. All formed elements circulate in fetal blood starting from week 13 of gestation. Lymphocytes predominate among blood leukocytes, because normally lymphocytosis is an inherent feature of blood. T cells and their subpopulations: active T lymphocytes, Tγ lymphocytes, and theophylline-sensitive and resistant cells are present. B lymphocytes are represented by cells with immunoglobulins M and G, zero cells, and large granular lymphocytes.

Key Words: *lymphocytes; blood; human fetus*

Embryogenesis of the immune system of a human fetus is an important aspect of immunomorphology. For a long time only maternal defense reactions were studied, while participation of the fetus in immunity reactions was doubted. New facts [1] about the immune system ontogenesis [11] in different organs and tissues [4] changed the situation. Lymphocytes occupy the central place in this system.

T and B lymphocytes were revealed in the sixties and seventies, and their cooperation in immune response was proved [5]. The subpopulations of T cells characterized by specific functions and supporting cellular mechanisms of immune regulation were identified. A special group of lymphocytes, zero cells, have no surface markers typical of T and B lymphocytes. These discoveries were made mainly in an adult organism, while fetal immunogenesis is little known; the fetus develops under sterile, but not antigen-free conditions. The development of fetal immune system depends on the maternal organism which creates special ecological conditions for the fetus. The lymphocyte count in the blood during different periods of embryogenesis is an indicator of fetal immune status. This fact is of both theoretical and practical significance in tissue therapy, which has been unfolded at present. Procurement of fetal donor material should include depletion of fetal lymphocytic components and selection of the periods of embryogenesis optimal for this purpose.

Our purpose was to study the counts and heterogeneity of human fetal blood lymphocytes at different periods of gestation.

MATERIALS AND METHODS

Blood specimens of 89 embryos and fetuses were obtained from healthy mothers at 4-40th weeks in maternity hospitals of Moscow. Fetal age was determined from the parietocalcanean size and weight of the fetus. Total population and subpopulations of T lymphocytes were recorded in the spontaneous lymphocyte rosette formation test with sheep erythrocytes and fetal proper erythrocytes [10]. Theophylline-sensitive and resistant lymphocytes [7], active T cells [9], and subpopulation of Tγ lymphocytes with suppressor-cytotoxic properties were distinguished [8].

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B lymphocytes with surface receptors to the complement C3 component were detected in the lymphocyte rosette formation test with bovine erythrocytes loaded with primary response antibodies and the complement. B lymphocytes with receptors to mouse erythrocytes were detected as described previously [10] and B lymphocyte subpopulation with membrane-bound IgM and IgG by the method described elsewhere [3]. Zero cell population was assessed as the difference between the total count of lymphocytes and the sum of total population of T lymphocytes and cells with receptors to the complement C3 component. Large granular lymphocytes were identified as described previously [6]. The relative and absolute counts of lymphocytes were estimated after statistical processing of the results (Tables 1-3).

RESULTS

Blood for precise quantitative analysis and isolation of lymphocytes was collected from fetuses starting from week 13 of gestation. Starting from this moment, all types of blood cells constantly circulate

TABLE 1. Counts of Lymphocytes, Zero Cells, and Large Granular Lymphocytes in Human Fetal Blood on Weeks 13-40 of Gestation(*M±m*)

Fetal age, weeks		Lymphocytes	Zero lymphocytes	Large granular lymphocytes
13-16 (*n*=5)	%	11.7±3.06	66.9±3.16	5.20±0.8
	10^9/liter	2.812±0.506	1.885±0.337	0.148±0.041
18-20 (*n*=6)	%	32.78±5.72*	54.4±6.80	8.4±2.30
	10^9/liter	4.703±0.521	2.528±0.448	0.381±0.118
21-23 (*n*=5)	%	56.67±4.55*	71.70±3.64	6.00±1.86*
	10^9/liter	11.918±2.018*	8.678±1.757*	0.731±0.236*
24-25 (*n*=3)	%	27.89±7.03	70.16±6.62	1.75±0.25*
	10^9/liter	5.012±0.96	3.609±0.975	0.100±0.034*
26-27 (*n*=8)	%	36.90±4.28	48.98±4.28	10.85±2.10
	10^9/liter	4.805±0.618	2.426±0.455	0.536±0.152
28-30 (*n*=6)	%	43.50±5.49	30.93±9.19	11.68±1.30
	10^9/liter	8.013±11.256*	1.949±0.683	0.847±0.153*
36-40 (*n*=13)	%	33.61±2.49	22.70±2.10	9.54±0.90
	10^9/liter	3.948±0.213	0.974±0.141	0.386±0.046

Note. Here and in Tables 2 and 3: *$p<0.05$ *vs.* the next group.

TABLE 2. Counts of T lymphocytes and Their Subpopulations in Human Fetal Blood on Weeks 13-40 of Gestation (*M±m*)

Fetal age, weeks		T lymphocytes	Active T lymphocytes	T lymphocytes with proper erythrocytes
13-16 (*n*=5)	%	13.10±1.60*	3.80±0.38	1.60±0.19
	10^9/liter	0.356±0.069*	0.105±0.019*	0.049±0.013
18-20 (*n*=6)	%	29.00±5.00	9.75±3.06	4.25±1.30
	10^9/liter	1.419±0.34	0.493±0.165	0.194±0.54
21-23 (*n*=5)	%	16.50±3.92	7.00±2.20	3.60±1.43
	10^9/liter	1.777±0.385*	0.734±0.157*	0.413±0.170
24-25 (*n*=3)	%	17.00±2.73*	5.67±0.68*	5.00±1.71
	10^9/liter	0.820±0.094*	0.275±0.023*	0.264±0.130
26-27 (*n*=8)	%	34.68±2.82	12.06±1.42	5.19±0.99
	10^9/liter	1.622±0.278	0.594±0.112	0.270±0.060
28-30 (*n*=6)	%	41.40±6.93	16.75±3.54	8.50±2.09
	10^9/liter	3.693±1.062	1.481±0.479	0.748±0.276
36-40 (*n*=13)	%	60.50±3.40	25.40±3.90	8.30±1.80
	10^9/liter	2.368±0.187	0.999±0.135	0.265±0.054

in the blood. Lymphocytes are the predominating type of leukocytes, i.e., lymphocytosis is a normal characteristic of fetal blood.

Blood lymphocytes were represented by T lymphocytes and their subpopulations: active T cells (Table 2), theophylline-sensitive and resistant Tγ lymphocytes. B lymphocytes include cells with receptors to the complement C3 component and to mouse antigens (Table 3). There were B lymphocytes with membrane-bound IgM and IgG, zero cells, and large granular lymphocytes (Table 1). The latter are a heterogeneous group, the majority of which are natural killer cells [2]. They possess natural cytotoxicity and are among the principal components responsible for antiviral and antitumor resistance of the organism. Prenatal changes in fetal blood large granular lymphocytes were so far unknown, which permits us to consider our results as original.

The count of each blood lymphocyte type changes with age (Tables 1-3). There is a general regularity in the time course of lymphocyte changes: by weeks 23-25 the counts of all blood lymphocytes dropped (Tables 1 and 2), including the predominating T cells, and by week 27 the count of these cells increases.

A high percentage of B lymphocytes (20%) secreting immunoglobulins is observed starting from week 13; then the count of these cells decreases and again rises by the 28th week.

Therefore, starting from the 13th week, all lymphocyte subpopulations are present in human fetal blood, including Tγ cells with suppressor-cytotoxic activity, zero cells, and large granular lymphocytes. The time course of lymphocytes and their subpopulations in fetal blood and their heterogeneity in fetogenesis reflect the development of the immunogenesis organs. High physiological lymphocytosis is typical of fetal blood as well as a high count of T lymphocytes. B cell immunity in fetal blood is more stable.

TABLE 3. Counts of B lymphocytes with Receptors to the Complement C3 Component and Mouse Antigens in Human Fetal Blood on Weeks 13-40 ($M \pm m$)

Fetal age, weeks		B lymphocytes with receptors to	
		complement C3 component	mouse antigens
13-16 (n=5)	%	20.00±1.60	16.50±1.40*
	10^9/liter	0.570±0.136	0.479±0.081
18-20 (n=6)	%	16.60±3.54	10.40±2.01
	10^9/liter	0.576±0.141	0.495±0.121*
21-23 (n=5)	%	11.80±1.34	17.10±3.44
	10^9/liter	1.463±0.393	1.972±0.410*
24-25 (n=3)	%	12.84±6.32	15.67±1.35
	10^9/liter	0.583±0.245	0.800±0.216
26-27 (n=8)	%	16.37±1.42	12.62±1.36
	10^9/liter	0.757±0.112*	0.593±0.119
28-30 (n=6)	%	27.67±5.64	18.83±3.64
	10^9/liter	2.371±0.698*	1.627±0.509
36-40 (n=13)	%	16.80±4.65	16.80±2.10
	10^9/liter	0.606±0.058	0.611±0.102

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