KINETICS OF ROSETTE-FORMING CELLS IN LYMPHOID ORGANS OF THE HUMAN EMBRYO AND FETUS

O. P. Ryabchikov

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Despite the rapid development of immunomorphology in the last 10-15 years, there are only occasional references in the accessible literature to the study of the time of appearance and kinetics of lymphocytes with surface markers of T-lymphocytes in human lymphoid organs during prenatal development [6-8, 12, 14, 15]. With the exception of the writer's previous publications [3, 5], no papers of this kind can be found in the Soviet literature.

The object of the present investigation was to study the relative percentage of lymphocytes forming large spontaneous rosettes with sheep's erythrocytes (E-RFC) and of lymphocytes forming large spontaneous rosettes with autologous erythrocytes (A-RFC) in cell suspensions of the thymus, spleen, mesenteric lymph nodes, red bone marrow, and liver during human prenatal development. Investigations were carried out on 126 human embryos and fetuses between 5.5 and 34 weeks of development, obtained from Moscow medical institutions from healthy mothers. The age of the embryos was determined from the length of the foot [1] and the age of the fetuses from the crown-rump and crown-heel lengths and body lengths. Suspensions from organs weighing under 20 mg and from mesenteric lymph nodes were obtained with the aid of a glass homogenizer in medium No. 199 followed by filtration through Kapron gauze. Suspensions of lymphocytes from organs with a larger weight were obtained by means of a liquid disintegrator. The cell suspensions thus obtained were washed three times with cold medium No. 199 at 1000-1500 rpm for 5-8 min, and the concentration was adjusted to between 2 and 4 million lymphocytes/ml. The number of dead cells in the test with 0.1% trypan blue solution did not exceed 5%. Suspensions of thymus cells before the 9th week of development and of spleen cells before the 13th week of development were not washed in order to avoid loss of lymphocytes, the number of which in these organs at these times of development is small. The reaction of spontaneous rosette formation of human lymphocytes with sheep's erythrocytes and with autologous erythrocytes was set by the method of Jondal et al. [10], with incubation of the cell mixture for 12-14 h at 4°C. Rosettes were counted in a Goryaev's chamber on 100-200 lymphocytes and the results were expressed in percentages. The specificity of the reaction of spontaneous rosette-formation was tested periodically by the rosette-abolition test with serum against human T-lymphocytes. The numerical data were subjected to statistical analysis on the Nairi-K computer.

EXPERIMENTAL RESULTS

The results are summarized in Table 1. It must be noted that the number of sheep's erythrocytes bound by thymus lymphocytes at the 7th-8th week of development does not exceed nine [3-9]. At the 9th-10th week of development of the thymus many rosettes consist of "morulas" and "semimorulas;" this may indicate intensive secretion and the powerful influence at this stage of thymosine, which cannot only activate receptors for sheep's erythrocytes on thymocyte precursor-cells [9], but can also considerably increase the number of these receptors [11]. The relative percentage of E-RFC in the thymus remained virtually unchanged from the 12th to the 34th week of development. The dynamics of development of T-lymphocytes in the human fetal thymus, thus revealed, was similar to that described in chick embryos [13]. It will be clear from Table 1 that human fetal thymocytes can bind autologous erythrocytes to form rosettes, and that the relative percentage of this subpopulation of thymocytes in-

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TABLE 1. Numbers (in %) of E-RFC and A-RFC in Cell Suspensions from Thymus, Spleen, Mesenteric Lymph Nodes, Red Bone Marrow, and Liver during Human Prenatal Development (M ± m)

Organ	Age of embryos and fetuses, in weeks													
	5½—6		7—8		9—10		11—12		13—15		16—20		21—34	
	E-RFC	A-RFC	E-RFC	A-RFC	E-RFC	A-RFC	E-RFC	A-RFC	E-RFC	A-RFC	E-RFC	A-RFC	E-RFC	A-RFC
Thymus	—	—	5,2±0,9	—	34,8±7,1	—	78,2±4,7 (68—90)	23	82,6±4,9	27	83,9±2,3	38,7±6.9	84,7±1,7	56,8±4,8
			n=4		*n*=5		*n*=5	*n*=1	*n*=5	*n*=2	*n*=19	*n*=6	*n*=43	*n*=16
Spleen	—	—	—	—	0	—	—	—	2,5±2,5	—	15.8±4,0	2,3±1,0	11,0±1,5	3,06±0,46
					n=2				*n*=3		*n*=13	*n*=3	*n*=44	*n*=16
Mesenteric lymph nodes	—	—	—	—	—	—	—	—	72	7	61,0±4,8	13,4±3,6	52,4±3,8	10,6±2,2
									n=1	*n*=1	*n*=8	*n*=5	*n*=22	*n*=14
Red bone marrow	—	—	—	—	0	—	—	—	0,8±0,4	1	1,8±0,6	0,8±0,4	1,5±0,2	1,3±0,4
					n=1				*n*=4	*n*=1	*n*=15	*n*=5	*n*=34	*n*=16
Liver	0,6±0,4	—	0,3±0,2	—	0,5±0,5	—	1,4±0,7	—	0,5±0,5	0	2,4±0,5	0,2±0,2	1,9±0,4	1,0±0,6
	n=5		*n*=13		*n*=4		*n*=6		*n*=4	*n*=1	*n*=17	*n*=5	*n*=31	*n*=9

Legend. n) Number of human embryos or fetuses investigated.

creases appreciably with the age of the fetus. The dynamics of E-RFC in the spleen showed considerable individual variations in the number of T-lymphocytes at each period of development studied. It is difficult at present to explain these data, for both the lymph nodes (Table 1) and the peripheral blood [6] at these times of development contain adequate numbers of E-RFC. However, other workers have shown [4] that the quantitative ratio between T_2- and T_1-subpopulations of T-lymphocytes differs in the spleen and lymph nodes (T_1 colonize chiefly the spleen, T_2 chiefly the lymph nodes). This fact suggests that in the course of human prenatal development there are individual variations in the order in which lymphocytes of these subpopulations leave the thymus to colonize the developing spleen, although the state of preparedness of the local microenvironment of the spleen to receive these groups of lymphocytes cannot be disregarded. The relative percentage of E-RFC in the mesenteric lymph nodes (Table 1) falls gradually during prenatal development with individual quantitative variations. It can be tentatively suggested that this decrease in the number of T-lymphocytes is connected with an increase in the overall weight of the lymph nodes and with the state of preparedness of the endothelial cells of the postcapillary venules of the newly formed lymph nodes to receive T-lymphocytes [2], which leads to redistribution of mature T-lymphocytes in these organs, for it has been shown that the T_2-subpopulation of T-lymphocytes, which colonizes mainly lymph nodes, consists of actively recirculating cells [4]. It will be clear from Table 1 that E-RFC were found in small numbers in the liver of 5.5-6 week embryos. At this period the thymus consists only of an epithelial anlage, and the process of its colonization with precursor cells begins only in the 7-week embryo. Asma et al. [6], who used specific antiserum against human T-lymphocytes, also found the latter in the fetal liver at these same times of development.

The experimental results thus suggest that during human prenatal development the number of E-RFC in the various lymphoid organs probably reflects the morphological and functional maturity of these organs. Among the total population of T-lymphocytes of human embryos and fetuses (E-RFC) there is a subpopulation of cells which binds autologous erythrocytes with the formation of large spontaneous rosettes. The E-RFC may appear in the human embryonic liver sooner than they are found in the thymus (5.5-6 weeks of development).

LITERATURE CITED

1. B. D. Abramov, "The study of the development of the gonads and female sex cells in early human embryogenesis," Author's Abstract of Candidate's Dissertation, Moscow (1976).
2. G. V. Kovalevskii, Outlines of Immunomorphology in a Model of Mycobacterial Sensitization of Animals Resistant to Tuberculosis [in Russian], Novosibirsk (1976).
3. R. M. Khaitov, in: General Problems in Pathology [in Russian], Vol. 5, Moscow (1977). p. 35.
4. Z. S. Khlystova, S. P. Shmeleva, O. P. Ryabchikov, et al., Byull. Éksp. Biol. Med., No. 7, 103 (1979).
5. G. E. M. Asma, W. Pichler, H. R. E. Schult, et al., Clin. Exp. Immunol., 29, 278 (1977).
6. S. Gupta, R. Pahwa, R. O'Reilly, et al., Proc. Natl. Acad. Sci., USA, 73, 919 (1976).
7. A. D. Hayward and Gunay Ezer, Clin. Exp. Immunol., 169 (1974).
8. G. S. Incefy, P. L'Esperance, and R. A. Good, Clin. Exp. Immunol., 19, 475 (1975).
9. J. Jondal, C. Holm, and H. Wigzell, J. Exp. Med., 136, 207 (1972).
10. D. B. Kaufman, C. R. Sledge, and M. Knust, Immunol. Commun., 6, 423 (1977).
11. N. Mukhopadhyay, D. I. Fernbach, D. M. Mumford, et al., Clin. Immunol. Immunopath., 10, 59 (1978).
12. M. Sugimoto, T. Yasuda, and Y. Egashira, Develop. Biol., 56, 281 (1977).
13. G. Wybran, M. C. Carr, and H. H. Fudenberg, J. Clin. Invest., 51, 2537 (1972).