# LYMPHOCYTE SUBPOPULATION COMPOSITION OF THE HUMAN LUNG DURING ANTENATAL DEVELOPMENT

**L. V. Van'ko, O. A. Timoshchuk, M. A. Nikolaeva, L. K. Romanova, and G. T. Sukhikh**

UDC 612.215.3:612.42]:612.646/.647

KEY WORDS: lymphocytes; fetus; lung.

The study of formation of defensive mechanisms in the human fetal lung at different stages of embryogenesis is of great interest, in particular, because of the high frequency of pathology of the respiratory organs in neonates. Several structural components of the lungs are involved in the development of local reactions of cellular and humoral immunity: broncho-associated lymphoid tissue (the BALT system), lymphoid cells of the lung parenchyma, and also the alveolar macrophages. The formation of the material substrate of local cellular immunity has been studied experimentally [5-7]. The results of an investigation of the subpopulation composition of lymphoid cells in the parenchyma of the adult human lung have been obtained [4]. No information on phenotypic characteristics of the lymphoid cells of the embryonic human lung could be found in the literature.

This paper describes a study of the subpopulation composition of lymphoid cells in the parenchyma of the human fetal lung at different stages of development.

## EXPERIMENTAL METHOD

Lungs were obtained from 25 human fetuses between the 8th and 28th week of prenatal development. Human embryos were obtained at abortions on medical grounds performed on healthy women, the fetuses as a result of spontaneous abortions from clinically healthy mothers, receiving no form of medication. The age of the human embryos was determined on the basis of foot length, that of the fetuses by comparing body weight, and crown-heel and crown-rump measurements with the aid of special correlation tables. In all cases the time of presumptive ovulation of the women was taken into account. Material for immunologic investigation was taken not later than 2 h after death of the fetuses, so as to avoid artefacts associated with post mortem changes in the cells. Lymphoid cells were isolated from the lung tissue by our modification of the method in [3]. Blood cells were removed by perfusion of the pulmonary vessels, and free-lying cells in the air passages were removed by irrigation of the lung. After careful removal of the trachea, bronchi, and large vessels, the lung tissue was cut into small pieces measuring 0.5-1.0 mm (at 4°C). The cell suspension was transferred into a conical centrifuge tube (50 ml), covered beforehand with embryonic calf serum to prevent adhesion of the cells, and containing 25 ml of medium with DNase (50 U/ml), and incubated with shaking on a water bath at 37°C for 20 min. After settling for 3-5 min the supernatant was poured off. The residue was treated with 10 ml of medium 199 and centrifuged at 450*g* for 1 min. The suspension was filtered through Kapron gauze into a siliconized tube and washed twice with cold medium 199. Lymphocytes were isolated from the cell suspension obtained from the fetal lungs and also from the umbilical cord blood of 30 newborn infants, by centrifugation on Ficoll–Paque (d = 1.077 g/cm) at 400*g* for 30 min (20°C). Cells from the interphase, after washing with medium 199, were diluted with buffered physiological saline (pH 7.4). Subpopulation analysis of the lymphocytes

All-Union Research Center for Maternal and Child Health, Ministry of Health of Russia. Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 626-629, June, 1992. Original article submitted October 28, 1991.

0007-4888/92/0006-0839$12.50  

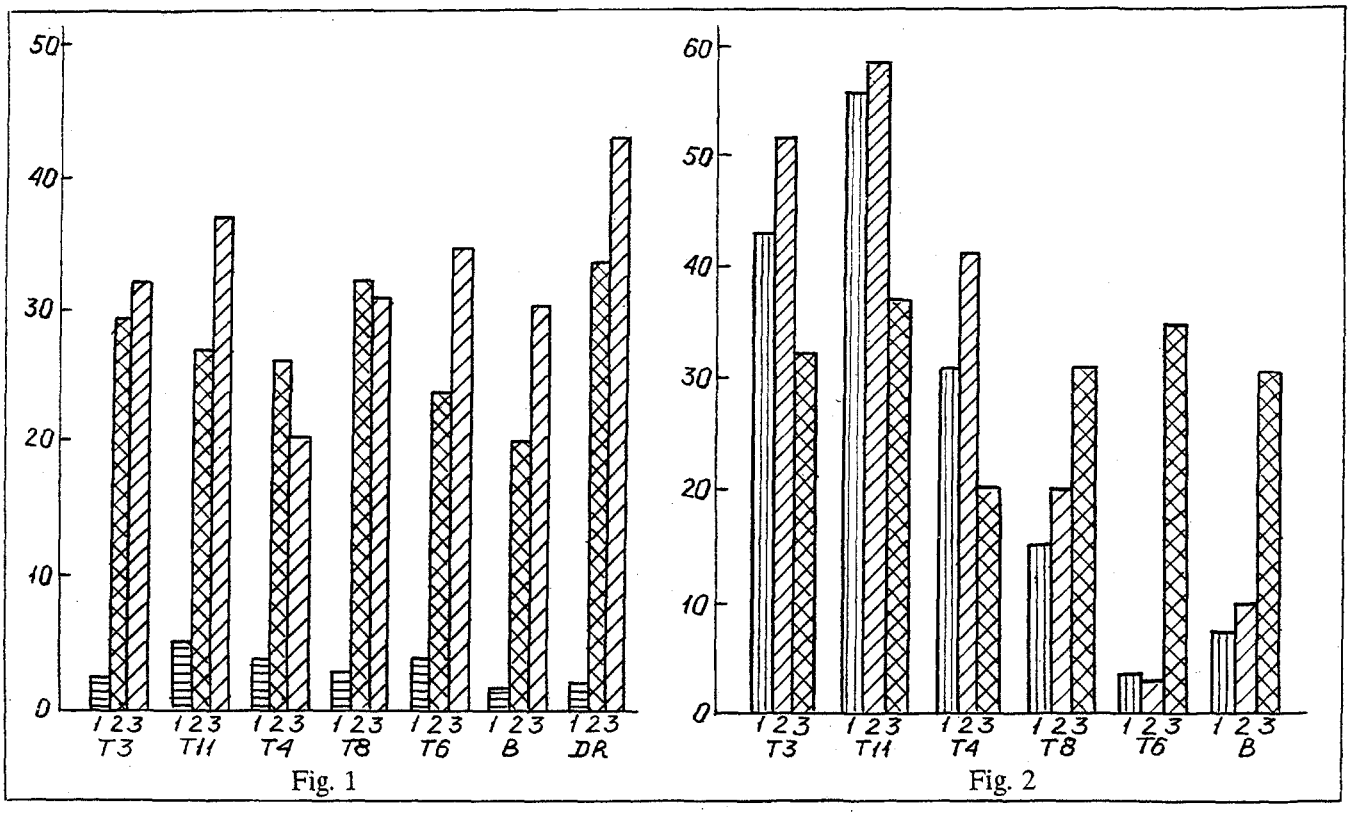


Fig. 1 Fig. 2

Fig. 1. Phenotypic characteristics of lymphocytes in human fetal lungs at different stages of intrauterine development: 1) 8-15 weeks, 2) 16-24 weeks, 3) 25-28 weeks of gestation. Here and in Fig. 2: ordinate, number of cells (in %).

Fig. 2. Phenotypic characteristics of lyphoid cells in lungs of a 25-28-week human fetus (3) and in blood of premature (1) and full-term (2) neonates. Abscissa, number of cells (%).

was carried out by flow cytometry on a "FacScan" cytofluorometer ("Becton Dickinson," USA), using fluorescently labeled monoclonal antibodies ("Ortho Diagnostic System" and Becton Dickinson, USA). To assess the cell cycle, a solution (5 mg propidium iodine, 0.1 g sodium citrate, 30 $\mu$l of the detergent Triton × 100 in 100 ml of distilled water) containing RNase in a concentration of 40 $\mu$g/ml was added to the cell suspension and the sample was incubated at 4°C for 15 min. Next, the DNA concentration in the cells was studied with a flow cytofluorometer, with analysis of the red fluorescence.

## EXPERIMENTAL RESULTS

Cells carrying markers of several subpopulations of T lymphocytes and B cells were found in the developing lungs in the early period of embryogenesis – at the pseudoglandular stage (Fig. 1). Cells expressing antigen T3 accounted for 2.5 ± 1.1% of the total in the lungs of an 8-week embryo. At the canalicular stage of lung development (15-24 weeks of gestation) the number of $OKT3^+$ cells was considerably increased, reaching 29.2 ± 9.5%, and it remained stable at the alveolar sac stage. The number of T lymphocytes expressing the OKT3 antigen in the human fetal lungs at the gestation times studied was similar to that in the liver, but differed significantly from their number in the spleen and thymus [2].

Lymphoid cells expressing T11 antigen were discovered in lungs of a human embryo, also by the 8th week of gestation. At the pseudoglandular stage of lung development, their number averaged 5.2 ± 1.4%, thus exceeding the relative percentages of other lymphocyte subpopulations. With an increase in the duration of gestation the number of $T11^+$ cells in the fetal lungs increased, to reach 26.9 ± 7.1% in the canalicular stage (16-24 weeks) and 37.0 ± 7.0% at the beginning of the alveolar sac stage (25-27 weeks). There is evidence that the number of $T11^+$ lympho-

TABLE 1. Number of Lymphoid Cells of Embryonic Human Lung at Different Stages of the Cell Cycle

| Phase of cell cycle | Gestation time, weeks | |
|---|---|---|
| | 8–15, n=7 | 16–27, n=3 |
| G1 | 69,1±6,1 | 78,6±7,5 |
| S | 29,4±5,8 | 20,6±7,2 |
| M+G2 | 1,7±0,4 | 0,7±0,3 |

cytes in umbilical cord blood of conventionally healthy premature infants at 35-37 weeks of development is 42.2% [1]. We obtained similar figures for $T11^+$ cells in cord blood of premature infants (in full-term neonates the percentage of these cells averaged 51.8 ± 4.2) (Fig. 2). Consequently, human fetal lungs at the beginning of the alveolar stage of development contain about the same number of $T11^+$ cells as the cord blood of conventionally healthy premature neonates.

Cells expressing antigen T6 were discovered in the lungs at the 9th week of intrauterine development. Their content at the pseudoglandular stage of development of the lungs averaged 3.9 ± 0.9%. The number of T6 lymphocytes was significantly increased at the canalicular stage of lung development, reaching 23.6 ± 5.4%. At the beginning of the alveolar sac stage, on average 34.8 ± 15.2% of $T6^+$ cells were identified in the lungs. It can be seen that during maturation of the lung the number of undifferentiated T lymphocytes did not decrease but, on the contrary, it increased. There were significantly fewer cells expressing this antigen in cord blood of premature and full-term neonates. This suggests continuous processes of differentiation of T lymphocytes in the developing lung, on the one hand, and the arrival of precursor cells in the lung, giving a population of $T6^+$ cells, on the other hand. The particular features of the microenvironment in the lung evidently correspond to differentiation in the direction: lymphoid precursor-cell – $T6^+$ lymphocyte.

The number of $T4^+$ and $T8^+$ cells (the principal subpopulations with immunoregulatory functions) at the pseudoglandular stage of lung development averaged 3.9 ± 0.9% and 3.0 ± 0.6% respectively. The number of $T4^+$ and $T8^+$ lymphocytes increased considerably at the canalicular stage, up to 26.3 ± 4.2% whereas the number of $T8^+$ cells rose to 32.1 ± 8.3%, although it remained stable at the later stages of lung development. Comparison of the relative percentages of cells of these populations in the lung with their numbers in the blood shows that the ratio of the regulatory subpopulations in the fetal lungs at the alveolar sac stage differs from the ratio of these subpopulations in cord blood, not only of full-term neonates, but also at an earlier period of development. However, the ratio between immunoregulatory subpopulations ($T4^+/T8^+$), even in the early stages of colonization of the lungs by lymphoid cells was similar to the ratio between these subpopulations in adult human lungs [4].

The presence of B lymphocytes in human fetal lungs, discovered at the 9th week of intrauterine development, reached on average 1.7 ± 0.7% at the pseudoglandular stage. At the canalicular stage the number was 20.1 ± 2.3% of B lymphocytes, and at the beginning of the alveolar sac stage, their number continued to rise, having reached 30.5 ± 22.8%. The time course of the change in the number of B lymphocytes in the lungs did not coincide with fluctuations of their number in the thymus, liver, spleen, and bone marrow or with the number of B lymphocytes in cord blood at late stages of gestation, and also in the adult human lungs, where, according to data in [4], only 9% of B lymphocytes was present.

It follows from our results that cells able to express class II MHC antigen at the pseudoglandular stage of lung development were present in comparatively small numbers (2.3 ± 0.8%). However, at the canalicular stage of lung development their number rose sharply, up to 33.6 ± 6.9%; this was followed by a further small increase at the beginning of the alveolar sac stage.

Determination of the fraction of cells present in the phase of DNA synthesis revealed a decrease in their number in the lung at the later stages of development (Table 1). The results indicate diminution of the proliferative capacity of the lymphoid cells of the lung during intrauterine development. Estimation of the fraction of DNA-synthesizing cells in cord blood revealed a much smaller number of these cells (4.7 ± 2.7%).

The presence of different subpopulations of lymphocytes in the tissue of the developing lung was thus found in 8-10-week human fetuses. A significant increase was found in the number of lymphocytes carrying various surface markers at the later stages of development, starting from the 17th-18th week of gestation. As the lung develops, the proliferative capacity of the lymphoid cells decreases.

## LITERATURE CITED


1. A. V. Samokhvalova, "Formation of the T lymphoid system in newborn infants in the early neonatal period," Author's Abstract of Dissertation for the Degree of Candidate of Science [in Russian], Moscow (1987).
2. G. E. M. Asma, R. L. Berch, and J. M. Vossen, Clin. Exp. Immunol., **53**, No. 2, 429 (1983).
3. P. G. Holt, B. W. Robinson, M. Reid, et al., Clin. Exp. Immunol., **66**, 188 (1986).
4. P. G. Holt, U. K. Kees, M. A. Shon-Hegrad, et al., Immunology, **64**, No. 4, 649 (1988).
5. M. Monieck, J. Glazier, and G. Hunninghake, Am. Rev. Resp. Dis., **135**, 72 (1987).
6. J. W. Simecka, J. K. Davis, and G. H. Cassell, Immunology, **57**, No. 1, 93 (1986).
7. S. P. Sorokin and R. F. Hoyt, Anat. Rec., **217**, 35 (1987).


# ROLE OF HISTAMINE RECEPTORS OF MOUSE AND GUINEA PIG PERITONEAL LEUKOCYTES IN THE PATHOGENETIC ACTION OF *Yersinia pestis* ADENYLATE CYCLASE


**B. N. Mishan'kin, L. E. Aseeva, L. A. Shevchenko, and B. D. Rublev**



UDC 616.155.3-008.94:175.824]-02:615.919:579.842.23

**KEY WORDS:** histamine receptors; mouse and guinea pig leukocytes; adenylate cyclase; *Yersinia pestis.*


The writers previously showed that $\beta$-adrenergic receptors of animal cells are involved in the pathogenetic action of the adenylate cyclase of *Yersinia pestis.* However, the group of histamine $H_1$ and $H_2$ receptors found on the surface of mixed lymphocytes and neutrophils remained unstudied. Considering the complex role of histamine in the development of infectious diseases and in the immune response, and its action on the cell through the adenylate cyclase–AMP system [10], we decided to study the possible role of peritoneal leukocyte histamine receptors of animals in the mechanism of the suppressive action of the recently discovered [1, 3] adenylate cyclase of *Yersinia pestis.*

## EXPERIMENTAL METHOD

Experiments were carried out on a purified preparation of *Y. pestis* adenylate cyclase with specific activity of 1700 pmoles $\cdot mg^{-1} \cdot min^{-1}$ [1]. Peritoneal leukocytes were obtained from albino mice and guinea pigs by the usual method. The chemiluminescence of the cell suspension was recorded as described previously [1]. Expression of $F^-$ and $C_3$ receptors (FcR and $C_3R$) was determined on guinea pig macrophages, by the rosette-formation method (EA-RFC) and chemiluminescence [5]. The kinetics of incorporation of $^{45}Ca^{2+}$ ("Izotop," USSR) was studied by methods in [2, 6]. Activity of $Ca^{2+}$-calmodulin-dependent protein kinase (CaM-PK) was determined as described in


Plague Research Institute, Rostov-on-Don. (Presented by Academician of the Russian Academy of Medical Sciences A. D. Ado.)


Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 629-631, June, 1992. Original article submitted October 8, 1991.




0007-4888/92/0006-0842$12.50