MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Maternal Aseptic Inflammation on the Structural Organization of Extraembryonal Organs and on the Proliferative Activity of Embryonic Liver and Lung Cells in Rats

S. I. Kolesnikov, Yu. V. Gladyshev, L. N. Demidova, N. G. Gladysheva, and S. V. Alisievich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 5, pp. 584-587, May, 1997
Original article submitted February 10, 1997

After 2 days of aseptic inflammation in pregnant rats the number of hematopoietic lineage cells in the embryonic liver decreased, while proliferative activity of cells in the embryonic lung increased. Degenerative changes were noted in the placenta.

Key Words: *inflammation; embryonic liver; embryonic lung; placenta*

Inflammation is a complex process that affects the internal organs and systems via cellular and secretory events. The effects exerted by pathological, including inflammatory, foci on cellular adhesion, chemotaxis, microcirculation, and other processes have been studied in sufficient detail [3]. Much less is known about cell proliferation in inflammation.

As we are aware, there is no data on proliferative processes in embryonic tissues under conditions of maternal inflammation, although the effect of maternal inflammation on the embryo is very important [5].

Turpentine has been generally used to model inflammation. Several hours after its injection a necrotic process develops at the injection site and passes through all stages of inflammation and regeneration [7].

In this study we examined structural changes in extraembryonal organs and DNA synthesis in embryonic liver and lung during aseptic inflammation in pregnant rats.

Institute of Pediatrics, East-Siberian Research Center, Siberian Division of the Russian Academy of Medical Sciences; Institute of Surgery, East–Siberian Research Center, Siberian Division of the Russian Academy of Medical Sciences, Irkutsk

MATERIALS AND METHODS

Aseptic inflammation was produced in 5 rats on day 18 or 19 of gestation by injecting turpentine into the back in a dose of 0.5 mg/kg body weight under sterile conditions. Control pregnant rats were injected with olive oil on the same days. All rats were killed on days 21-22 of gestation, extraembryonal organs (placenta and yolk sac) and embryonic organs (liver and lungs) were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4), postfixed in 1% OsO_4, and embedded in Epon-Araldite by the standard procedure. For autoradiographic examination, pieces of embryonic liver and lung were incubated for 60 min at 37°C in medium 199 containing ^{3}H-thymidine in a concentration of 1 mBq/ml. After incubation, they were fixed in 10% paraformaldehyde, washed for 24 h with repeated changes of the buffer, embedded in Epon-Araldite and used to prepare semithin sections (2 μ) which were coated with type M emulsion, incubated for 3 weeks at 4°C, developed, and stained with toluidine blue. Labeled nuclei were counted at least per 1000 cells, and the nuclear

0007-4888/97/0005-0509$18.00

TABLE 1. Morphometric Parameters of Embryonic Liver 48 h after Turpentine Administration ($M \pm m$)

Parameter	Control group	Test group
Area of hepatocyte nucleus, arb. units	0.66±0.16	0.8±0.75
Area of hepatocyte cytoplasm, arb. units	2.37±0.31	2.65±0.36
Number of cells per field of view:		
hepatocytes	7.32±1.7	7.1±0.87
hematopoietic cells	18.2±3.5	11.15±1.61*
NLI of embryonic liver cells:		
hepatocytes	3.4±0.25	4.1±0.41
hematopoietic cells	11.6±1.21	5.36±0.8*
NLI of embryonic lung cells:		
mesenchymal cells	7.8±0.8	12.7±1.76*
pneumocytes	8.3±1.4	14±2.3*

Note. *$p<0.01$ compared with the control group.

labeling index (NLI) was calculated and expressed in percent. Since it was impossible to distinguish between first- and second-order pneumocytes at the light microscopic level, mesenchymal cells and pneumocytes were considered together. Morphometric analysis of the structural organization of embryonic liver was carried out on toluidine blue-stained semithin sections. Nuclear and cytoplasmic areas of hepatocytes and the relative proportions of hepatocytes and cells of the hematopoietic lineage were calculated.

The significance of quantitative intergroup differences was evaluated by Student's t test.

RESULTS

Two days after turpentine injection, the second and (in some cases) third trophoblast layers were dilated. The nuclei and cytoplasm of trophoblast cells were often vacuolated (Fig. 1, *a*). The placental sinuses contained increased numbers of erythrocytes, and the areas of destruction as well as necrotic cells were seen in the intermediate zone of the placenta. The only notable change in cells of the yolk sac was the presence of clarified areas in their apical part. The overall structure of the embryonic liver in the test rats was preserved. Hepatocytes, as in normal liver, were not oriented around vessels, and hematopoietic cells were arranged in variously sized islets among hepatocytes. Nuclear and cytoplasmic areas did not differ significantly from their control values, but the number of hematopoietic cells was significantly reduced (Table 1).

Autoradiographs showed nonuniform ^{3}H-thymidine incorporation into the embryonic liver in the control group. Most DNA precursors appeared were incorporated into hematopoietic cell nuclei (Table 1). In experimental group ^{3}H-thymidine incorporation into the embryonic liver was significantly decreased because of a reduction in the number of hematopoietic cells (Fig. 1, *b*). On autoradiographs of embryonic lungs in the control group, silver grains were arranged in a uniform manner above the nuclei of mesenchymal cells and pneumocytes. In the test group, labeled cells, both mesenchymal and pneumocytes, were present in significantly increased numbers (Fig. 1, *c*).

There is considerable body of evidence regarding the influence of various factors on embryonic hematopoiesis and lung development. It was found, for example, that chronic prenatal hypoxia increases the area of hematopoietic foci [2]. Augmented hematopoiesis was also noted after administering insulin to pregnant animals [8].

Of considerable interest and importance is our finding of increased proliferative activity of embryonic lung cells in experimental group, given that enhancement of DNA synthesis results in activated protein-synthesizing processes in the cell. Embryonic fibroblasts may be capable of producing biologically active substances that accelerate maturation of secondary pneumocytes. Injection of proline analog into pregnant rats inhibits fibroblast proliferation in fetal lungs [8], which leads to a decrease in the synthesis of surfactant and delayed formation of alveoli.

The ratio of hepatocytes to hematopoietic lineage cells changes from one day of gestation to the next [1], and a decrease in the number of hematopoietic cells can serve as a reliable indicator of accelerated fetal maturation, since the liver performs hematopoietic function only during embryonic and early postnatal periods. Reduced numbers of DNA-synthesizing cells in the embryonic human liver have also been observed clinically in cases of maternal disease [6].

Thus, maternal inflammatory processes during pregnancy may cause structural changes in extraem-

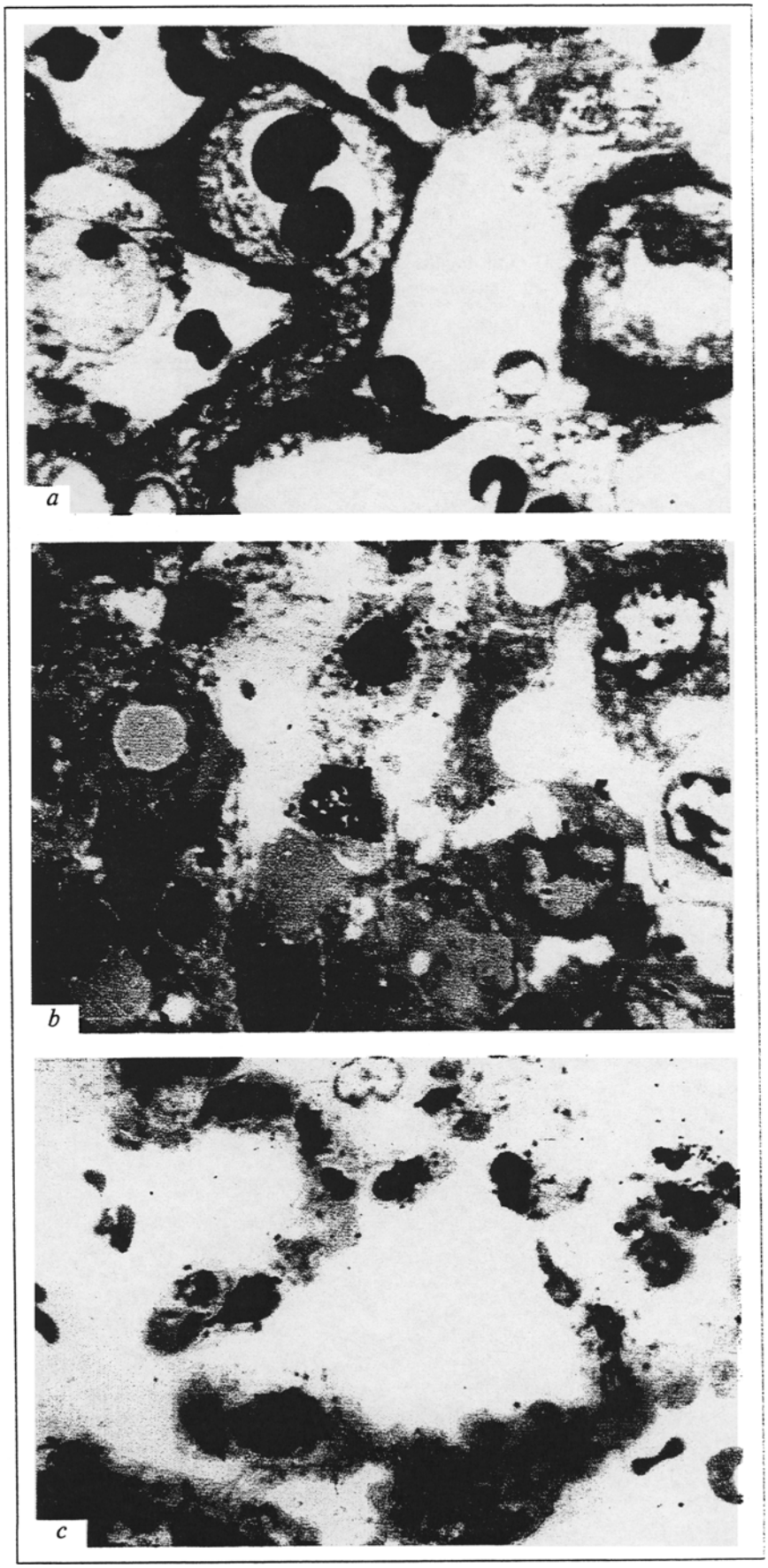

Fig. 1. Dilated and vacuolated trophoblast (*a*), lowered proliferative activity of embryonic liver (*b*), and increased ^{3}H-thymidine incorporation into embryonic lung cells (*c*) 48 h after turpentine administration. Semithin sections stained with toluidine blue. *a*, *b*) ×1000; *c*) ×400.

bryonal organs and reduce the number of hematopoietic cells in the embryonic liver while activating proliferative processes in the embryonic lung, apparently as a result of fibroblast activation. The latter observation possibly reflects an earlier maturation of embryonic tissues when inflammation occurs in the mother's body.

REFERENCES

1. V. I. Grishchenko, G. S. Lobyntsev, I. A. Votyakov, *et al.*, *Hematopoietic Cells of the Embryonal Liver* [in Russian], Moscow (1989).
2. A. I. Utkina and S. S. Timoshin, *Byull. Eksp. Biol. Med.*, **110**, No. 1, 541-543 (1990).
3. A. M. Chernukh, *The Infectious Focus of Inflammation* [in Russian], Moscow (1965).
4. I. Y. R. Adamson and G. M. King, *Lab. Invest.*, **57**, 439-445 (1987).
5. J. Ph. Babala, *J. Gynecol. Obstet. Biol. Reprod. (Paris)*, **7**, 829-835 (1985).
6. J. A. Mochage, J. H. Janssen, and J. C. Franssen, *J. Clin. Invest.*, **79**, 1635-1641 (1987).
7. R. Vrancks, S. L. Cohen, and A. M. Maya, *Inflammation*, **13**, 79-90 (1989).
8. J. A. Widness, J. B. Susa, and J. F. Garcia, *J. Clin. Invest.*, **67**, 637-642 (1981).