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DISORDERS OF ERYTHROPOIESIS DURING ACUTE AFRICAN SWINE FEVER

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It has been previously shown that the virus of acute African swine fever (ASF) infection causes significant alterations in the population of white blood cells. Thus, it has been assumed that ASF disrupts hematopoietic homeostasis through an unknown mechanism. To test this assumption, we have conducted this study to evaluate the changes of red blood cells (RBCs) in peripheral blood during experimental ASF virus infection. Light microscopy of erythropoiesis in this case showed distinct features of dyserythropoiesis. From the beginning of infection, juvenile forms of RBCs, such as the largest cells of erythron, rubriblasts, were observed in the peripheral blood of infected pigs. Among the erythroid precursors, up to 60% of all cells were binucleated, which indicates that acute ASF infection is accompanied with the emergence of pathological forms of RBCs.

***Keywords*:** African swine fever (ASF), red blood cells (RBCs), erythropoiesis, erythroid precursors, infection, hemolysis.

Introduction. African swine fever (ASF) is an acute highly contagious disease characterised by viremia. The pathogen DNA-containing virus of the genus African swine fever virus (ASFV) of the family of *Asfaviridae* has high virulence. Reproduction of the virus occurs in lymphoid and myeloid tissues of the immune system, macrophages of mononuclear phagocytes and is accompanied by cytopathic effects on lymphocytes, monocytes, macrophages and endothelial cells [1]. Due to the defeat of macrophages and endothelial vascular necrosis, the permeability of blood vessels dramatically increases, appears hyperaemia, thrombosis and massive hemorrhage in the skin and in all internal organs [2]. As we have shown in our previous studies, in acute form of ASF are also detected abnormalities in erythropoiesis [3]. Alteration of erythropoiesis at acute ASF is manifested as a decrease in the number of red blood cells in peripheral blood, the appearance of microcytes that reach almost the half of all red blood cells (RBCs) at the peak of the disease. Another sign of the disorders of erythropoiesis from the first day of the disease is the appearance of nuclear forms of RBCs. In the present study we have investigated the main indices in nucleated forms of RBCs in experimentally induced acute form of ASF.

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Materials and Methods. In our study we used nine healthy pigs of the same weight (30–35 *kg*) and age (6 month old) for infection and control. Animal care and euthanasia were done according to Guide for the care and use of Laboratory Animals, AVMA Guidelines on euthanasia and local guideline for animal care and use.

We have used ASFV (genotype II), which has hit Georgia, and then the neighboring countries, including Armenia as well [4]. Determination of infectious titer was performed by ASF hemadsorption [5]. To determine the infectious titers of virus, blood was taken from ophthalmic vein of the infected pigs, in plasma of which hemadsorption titers of virus were determined.

ASFV used in experiments on pigs, amounted to 10^4 hemadsorption units HADU50/*mL*. For intramuscular injections, 10^4 hemadsorption units (HADU50)/*mL* of ASFV (genotype II) were injected into nine pigs for further study. Virus titration for each day post-infection was carried out as described previously [6] and expressed as hemadsorption units (HADU50).

Peripheral blood for preparation of smears was taken from ophthalmic veins of healthy and infected pigs each day. Smears were fixed in 96% ethanol and stained with azure eosin by Giemsa. The classification of RBCs was performed as described previously [7].

Results and Discussion. From the early stages of infection nucleated RBCs that were various forms of early (basophilic) and late (metarubricytes and polychromatophilic rubricytes) erythroid precursors appeared in the blood of pigs. The number of nucleated RBCs did not exceed 0.5% of all RBCs. Early erythroid precursors in peripheral blood arose from the beginning of infection (Table). At 1 DPI, early erythroid precursors were represented by prorubricytes, whose sizes varied from 11 μm to 13 μm (Fig. 1, 2).

Changes detected in the population of nucleated RBCs during acute ASF infection

DPI	Nucleated cells (– none; + slight; ++ moderate; +++ abundant)				
	metarubricytes	polychromatophile rubricytes	basophilic rubricyte	prorubricyte	rubriblasts
0	–	–	–	–	–
1	+	+	–	+	–
2	+	+	–	+	–
3	+	+++	+	+	+
4	+	++	+	+	+
5	+	+	+	+	+
6	+	–	–	+	+
7	+	–	–	+	–

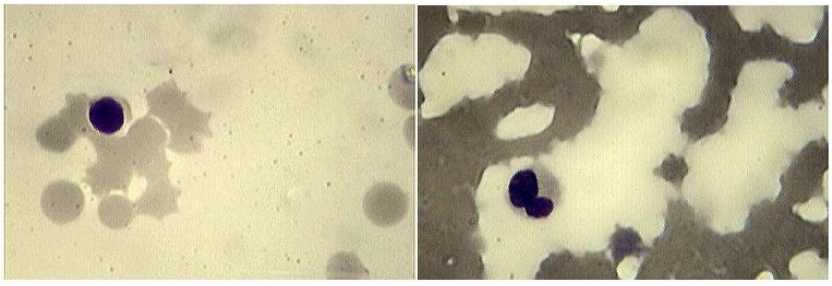
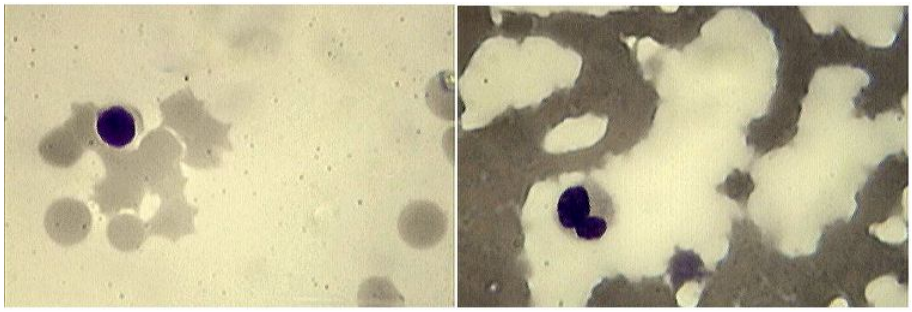


Fig. 1. Metarubricytes with additional nuclei. Magnification ×1000.



a b

Fig. 2. a) Polychromatophile rubricyte; b) polychromatophile rubricyte with additional nuclei. Magnification × 1000.

Basophilic rubricytes (Fig. 3) were observed at 3 DPI and remained detectable up to 5 DPI.

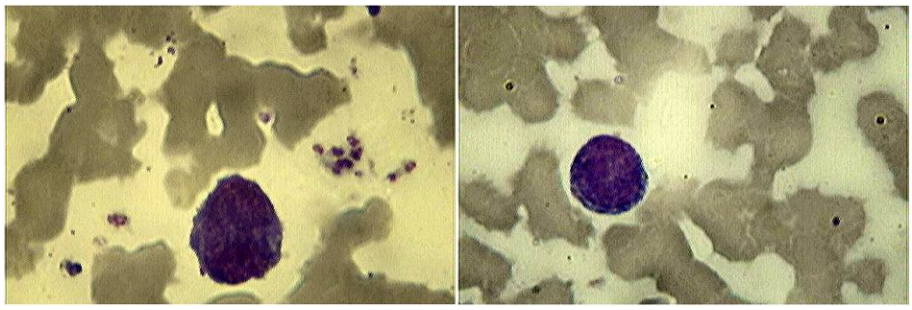


Fig. 3. Prorubricyte and basophilic rubricyte. Magnification ×1000.

The largest cells of erythron (rubriblasts) were detected from 3 DPI and absent by 7 DPI. Their size was mainly less than 20 μm and they contained a large nucleus with one or two nucleoli.

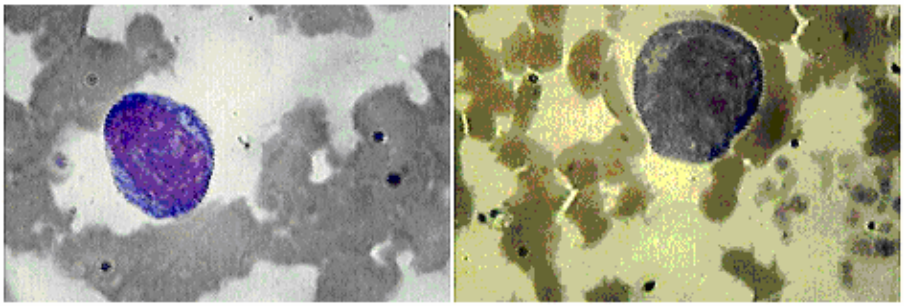


Fig. 4. Rubriblasts. Magnification ×1000.

Although metarubricytes (Fig. 1) and a small number of polychromatophilic (Fig. 2) rubricytes were clearly observed at 1 DPI, the bulk of late erythroid precursors in peripheral blood were mainly comprised of polychromatophilic rubricytes, as well as fewer metarubricytes (Table).

Surprisingly, no polychrometophilic rubricytes were detected in the final phase of infection. The average size of late erythroid precursors was slightly smaller than the size of these cells in the bone marrow of healthy pigs (unpublished data). It is important that, among the late erythroid precursors, up to 60% of all cells had additional nuclei (Fig. 1, 2), particularly in the population of metarubricytes, where this phenomenon was observed for up to 80% of cells (data not shown).

We have previously reported that ASFV (genotype II) infection is accompanied with profound changes in white blood cells, indicating that hematopoietic homeostasis is disrupted in ASF-infected pigs [8]. Herein, our data demonstrate that acute ASF infection also leads to serious alterations in the population of circulating RBCs.

There are two main mechanisms, which can explain appearance of early precursors of erythropoiesis in blood during the acute ASF. The first mechanism is the loss of RBCs via hemolytic processes during ASF. Erythropoiesis, the bone marrow production of erythrocytes by the proliferation and differentiation of hematopoietic cells, replaces the daily loss of 1% of circulating erythrocytes that are senescent [9]. In case of increased loss of RBCs the early precursors of erythropoiesis can be removed from bone marrow to the peripheral blood.

The second mechanism of immature RBCs arising depend on direct macrophage damaged by ASF. The documented studies of the pathogenesis of ASF in domestic swine suggest that the virus mainly infects cells of the reticulo-endothelial system, the macrophages being essentially the cells allowing a productive infection [6]. There are several examples in literature showing that macrophages not only promote erythropoiesis by providing iron, but also by directly stimulating proliferation and survival of erythroblasts.

Conclusion. The current study points to a disturbance of erythropoiesis, which is manifested by arising of nucleated, early (basophilic) and late (metarubricytes and polychromatophilic rubricytes) erythroid precursors also appeared, binucleated erythroid precursors have been observed, indicating that acute ASF infection is accompanied with the emergence of pathological forms of RBCs in the host blood. These results reinforce the statement that haematopoiesis undergoes significant changes during infection. Further experiments will be required to determine whether ASF affects the erythropoiesis via hormones/cytokines that are thought to be involved in erythropoiesis and regulated by ASF [10], as well as to determine the nature of binucleation. This and further studies may increase our understanding of the pathology of ASF.

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