PRIMATOLOGY

Experimental Study of Ebola Hemorrhagic Fever in *Papio hamadryas*

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The course of disease caused by Ebola virus in *Papio hamadryas* is studied by clinical and biochemical methods. The severity and outcome of the disease are found to be unrelated to the degree of viremia. The development of deep lesions in the liver and kidneys in the course of the disease, as well as of a disseminated intravascular coagulation syndrome with an abnormally prolonged hypercoagulation stage is demonstrated.

Key Words: *Ebola virus; monkeys; disease; blood*

Among the hemorrhagic fevers the disease caused by Ebola virus is remarkable for its high mortality rate, grave course, and frequent development of a disseminated intravascular coagulation syndrome [7,8]. The aim of this study was to trace the course of hemorrhagic Ebola fever in *Papio hamadryas* using clinical and biochemical methods.

MATERIALS AND METHODS

The Zaire strain of Ebola virus was obtained from the Belarus Research Institute of Epidemiology and Microbiology, Ministry of Health of Belarus. Thirteen *Papio hamadryas* from the Sukhumi breeding center were infected subcutaneously in doses of 20 to 50 LD_{50} for these animals. All the manipulations with the virus and infected animals were carried out in special rooms with the P4 level of protection. Before sample collection the animals were narcotized with subcutaneous calypsol in a dose of 0.5 ml/kg body weight. Blood was collected from the superficial veins of the upper and lower limbs.

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Before inoculation and daily thereafter the animals were examined, their body temperature was measured, and blood samples were collected. Red cells, leukocytes, and platelets were counted and the red cell sedimentation rate and hemoglobin level were assessed by routine methods [3]. Measurements of total protein and protein fractions, β-lipoproteins, bilirubin, creatinine, urea, malonic dialdehyde (MDA), C-reactive protein, circulating immune complexes (CIC), activities of glutamic oxalacetic and pyruvic transaminases in the serum, and the thymol test were carried out [2]. The status of the blood clotting system was assessed by the platelet count, thrombin and prothrombin index, ethanol test, and content of fibrinogen and fibrin degradation products [6]. The absolute and relative counts of T and B lymphocytes and the phagocytic activity of neutrophils toward zymosan were assessed [5].

RESULTS

Preliminary studies on 6 intact animals showed that blood sampling during 14 days did not appreciably influence any of the parameters tested;

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TABLE 1. Changes in Serum Biochemical Parameters of Baboons Infected with Ebola Virus ($M \pm m$)

Parameter	Day of observation					
	0	3[1]	5	7	8	9
Glutamic–oxalacetic transaminase, units act.	0.6±0.1	0.9±0.1	1.1±0.1*	1.8±0.2*	2.2±0.3*	2.7±0.8*
Glutamic–pyruvic transaminase, units act.	0.6±0.1	1.0±0.2	0.9±0.2	2.7±0.5*	2.8±0.4*	2.7±0.8*
Bilirubin, μmol/liter	4.9±0.5	10.4±1.7*	6.9±1.2	9.1±0.9*	28.4±8.3*	20.0±4.0*
Thymol test, units S/HO	2.1±0.2	4.4±0.8*	2.6±0.4	3.0±0.7	3.9±0.8	2.8±0.8*
Urea, mmol/liter	5.3±0.4	5.4±0.4	6.8±0.6	11.9±0.9*	17.6±3.6*	28.4±5.5*
Creatinine, mmol/liter	156.4±6.6	149.1±9.2	167.0±6.9	256.7±27.8*	335.0±67.5*	363.0±88.4*

Note. Here and in Table 2: the asterisk shows reliable differences from the parameters on day 0, $p<0.05$. [1]The values of all parameters on days 0 and 1 postinoculation do not reliably differ, except for bilirubin, which increases to 7.2±0.5.

there was just a tendency for the red cell count to drop from $4.5\pm0.1\times10^{12}$/liter at the beginning of the experiment to 3.7±0.3 at the end. The values of the parameters in intact baboons did not differ from those known for these animals [4].

In 50% of animals Ebola virus can be detected in the blood 1 to 1.5 days before the onset of fever. The virus was detected by biotitration on suckling mice on day 4 (2.9±0.4 log LD_{50}) and by the plaque method in a Vero cell culture on day 5 (3.8±0.4 log PFU/ml of blood). The titer of the virus in the blood increases as the infection progresses to 6.0±0.4 log PFU/ml on days 7-9. No relationship was detected between the Ebola virus level in the blood, the disease severity, and the time of death.

The onset of the disease in baboons infected with Ebola virus is acute, with fever of up to 40.5-41.8°C. The mean duration of the incubation period was 6.3±0.3 days and the mean life span 8.5±0.2 days. Fever lasted for about 2 days, after which the animals died. Four to six hours before death the body temperature dropped to 35.5-36.5°C. In some cases there was no fever, and the infection culminated in death against the background of pronounced hemorrhagic symptoms. The acute phase is characterized by hyperthermia, adynamia, anorexia, weakened aggressiveness, and conjunctivitis, sometimes with tremor of the limbs in the terminal stage. The disease was attended by the hemorrhagic syndrome: 50-60% of animals developed maculopapulous eruptions on the skin of the hands and feet, spreading to the chest, abdomen, and face, and tending to merge in the terminal stage. Bloody vomitus, nasal bleeding, and melena were observed in some baboons.

Regional lymph nodes were enlarged and compact in dead monkeys. The liver was enlarged,

TABLE 2. Time Course of the Blood Coagulation System in Monkeys Infected with Ebola Virus ($M \pm m$)

Day	Parameter				
	thrombin index, %	prothrombin index, %	fibrinogen content, g/liter	ethanol test, arb. units	content of fibrinogen degradation products, arb. units
0	100.0±0.0	100.0±0.0	3.4±0.4	0.0±0.0	0.2±0.1
1	105.8±6.7	104.9±6.8	5.3±0.5	0.2±0.1	0.3±0.1
2	105.3±10.5	139.5±15.2*	5.0±0.6	0.1±0.1	n.d.
3	123.5±12.8	142.7±15.0*	4.0±0.4	0.4±0.2	0.1±0.1
4	148.0±15.5*	156.6±16.9*	4.3±0.4	0.5±0.2	n.d.
5	137.9±26	141.2±14.7*	4.5±0.4	0.3±0.1	0.4±0.1
6	103.5±9.2	112.7±12.1	3.7±0.4	0.2±0.1	0.3±0.1
7	74.9±7.2*	75.7±6.9*	3.9±0.6	1.0±0.3*	1.7±0.3*
8	63.8±0.6*	62.8±15.0*	3.0±0.6	0.3±0.2	3.0±0.0*
9	20.1±4.6*	32.3±6.6*	1.8±0.3*	0.0±0.0	1.5±0.5*

Note. n.d. = not determined.

loose, and plethoric, with 2-3-cm yellowish foci which often merged; sometimes the whole liver was a clayey yellow. The spleen was enlarged, plethoric, and bluish purple, with a strained capsule. There were signs of bleeding from the nose and mouth and hemorrhages in the large and small intestine walls.

The results indicate that changes in the hematologic parameters as a rule coincide with the onset of clinical manifestations of the disease. The red cell sedimentation rate increased threefold on day 5 and more than sixfold on days 6-7. Thrombocytopenia was first detected on day 7 and progressed till the moment of death. The leukocyte count increased from 13.5±1.4 to 20.5±0.6×10^9/liter on days 6-7, with the formula shifting to the left due to immature forms. These changes intensified right up to the end of the experiment. The percentage of T and B lymphocytes was stable in the course of infection, but in the terminal stage their absolute counts dropped. The red cell count, nonspecific activation of phagocytes, and phagocytic index did not reliably change over the entire course of the disease.

The time course of the biochemical parameters is presented in Table 1. The marked rise of the levels of urea and creatinine on day 7 points to the development of acute necrotic processes in the renal tissue. The increased level of bilirubin and changed transaminase activity reflect dysfunction of the liver, which is the main target organ in Ebola hemorrhagic fever. The time course of the thymol test and of β-lipoproteins indicates disorders in hepatic function characteristic of acute hepatitis. The content of total protein and the levels of CIC and MDA did not noticeably change in the course of infection. The increase of the level of C-reactive protein (from 0.2±0.1 to 0.8±0.1 arb. units on day 2 to 0.9±0.2 arb. units on day 7) in parallel with a rise in the α_1-, α_2-, and γ-globulin fractions (from 5.1±0.5 to 15.8±4.6%; from 9.5±0.4 to 23.3±4.6%; and from 16.0±0.9 to 22.8±2.3% on day 9, respectively) and the moderate β-lipoproteinemia (from 42.1±4.1 to 131.4±29.8 arb. units on day 8) point to the appearance of acute-phase proteins and to activation of the complement system. The albumin/globulin ratio fell from 1.2±0.1 to 0.8±0.1 on day 5 and to 0.2±0.1 on day 9.

Study of the parameters of the blood clotting system (Table 2) showed that as early as on day 2 postinoculation hypercoagulation develops, reaching its maximum by day 4. On day 6 the parameters approach the normal values, and then drop, this leading to increasing hypocoagulation right up to death. This data permit us to speak of the development of the disseminated intravascular coagulation syndrome with an abnormally prolonged hypercoagulation stage characterized by very high coagulation parameters in parallel with a relatively low level of fibrinogen [1]. The hypocoagulation stage was poorly expressed, rapidly progressing to the terminal stage.

Analysis of the data indicates that the course of the disease in baboons infected with Ebola virus is similar to that in humans [9].

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