PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Damage to the Internal Organs of Experimental Animals Infected with Marburg's Virus

E. I. Ryabchikova, L. A. Vorontsova, A. A. Skripchenko, A. M. Shestopalov, and L. S. Sandakhchiev

UDC 578.833.2:616:576.31

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 4, pp. 430-434, April, 1994
Original article submitted November 16, 1993

It is shown that in grivets and guinea pigs Marburg's virus reproduces in cells of the mononuclear phagocyte system, in hepatocytes, and in a few endotheliocytes. Marked pathological changes develop in the liver, spleen, and kidneys. A peculiarity of infection in monkeys is the entire absence of morphological manifestations of an inflammatory reaction and of immune system activation. Cells of the mononuclear phagocyte system are thought to play a crucial role in the development of pathological changes in the organism of infected monkeys and guinea pigs.

Key Words: *Marburg's virus; monkeys; guinea pigs*

Research into the course of disease in different types of experimental animals is one way of studying the pathogenesis of various diseases. *Cercopithecus* hemorrhagic fever (Marburg's disease) is one of the least studied diseases. Lower monkeys and guinea pigs are used as experimental models of this disease. In grivets the development of infection goes along with severe damage to the liver, kidneys, and spleen; reproduction of Marburg's virus (MV) occurs in hepatocytes (Hc), in the endothelium, and in the cells of the mononuclear phagocyte system (MPS) [3]. No such data are available in the case of guinea pigs.

The aim of the present research was to perform a comparative study of MV reproduction and of the damage to organs in grivets and guinea pigs over the time course of the disease.

Vektor Industrial Center, All-Russia Research Institute of Molecular Biology, Novosibirsk

MATERIALS AND METHODS

MV (Popp strain) obtained at the L. A. Tarasevich State Institute for Research and Standartization of Medical Biological Preparations underwent 9 passages in guinea pigs. A 15% homogenate of the liver of guinea pigs which were infected with a dose of 100 LD_{50} was used in the study. The initial virus titer was 7.8 log LD_{50}. Grivets (mature male monkeys weighing 4-5 kg) and nonpedigree guinea pigs of both sexes weighing 250-300 g were obtained from the vivarium of the All-Russia Research Institute of Molecular Biology. The animals were intraperitoneally infected with MV in a dose of 100 LD_{50}. Samples were obtained over 8 days starting at 24 h postinfection; for this purpose, every day three guinea pigs and one monkey were killed under chloroform. The virus content in the blood and liver homogenate (15%) was measured in guinea pigs by the method of end-point dilutions (LD_{50}/ml). For microscopic examination

0007-4888/94/0004-0429$12.50

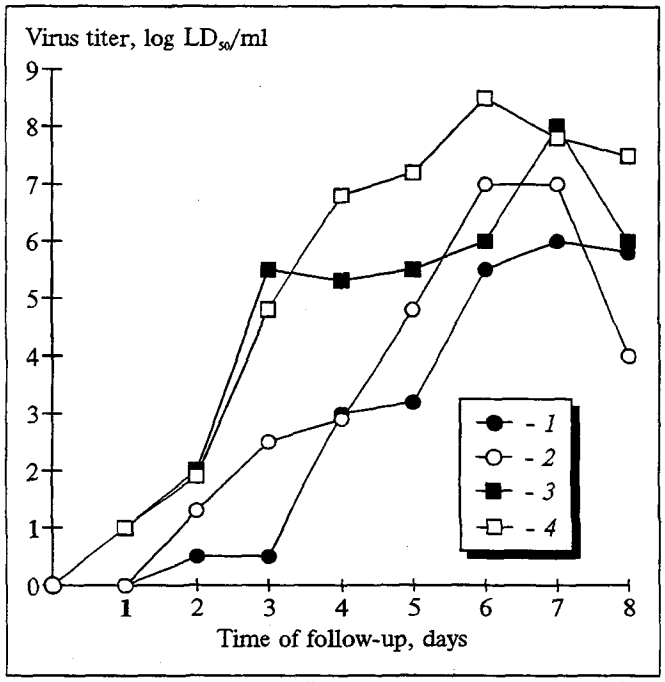

Fig. 1. Accumulation of MV in experimental animals. *1*) in liver of grivets; *2*) in blood of grivets; *3*) in liver of guinea pigs; *4*) in blood of guinea pigs.

samples of the liver, spleen, kidneys, lungs, and of the inguinal and mesenteric lymph nodes of monkeys and guinea pigs were fixed in a mixture of 2.5% glutaraldehyde and 4% paraformaldehyde (1:1) during 2-5 days. The preparations were postfixed with 1% osmium tetroxide, dehydrated in ethanol and acetone, and embedded in Epon-Araldite. Semithin sections were stained with Azure-2. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under JEM-100 S (Jeol) and H-600 (Hitachi) electron microscopes.

RESULTS

Guinea pigs and monkeys intraperitoneally infected with MV died on days 7-8. Disease in both species was accompanied by virus accumulation in the blood and liver (Fig. 1).

Our study demonstrated that throughout the course of investigation the severity of damage to the organs was higher in grivets than in guinea pigs. The major target tissue in Marburg's disease is the liver [3,4]. On days 1-3 postinfection, marked vascular congestion, vacuolarization of Hc, and disappearance of glycogen in Hc were observed in monkeys. In guinea pigs damage to the liver was not marked during the first 3 days: a small fraction of the hepatic veins and sinusoids were plethoric; scant clusters of Hc with light cytoplasm were observed near the central veins.

MV multiplication in the liver of both animal species (in Hc and Kupffer cells) was not detected by electron microscopy till day 4 (Fig. 2, *a*). Infected cells were solitary; Hc were grouped near the central veins. Particles exhibiting the shape characteristic of MV were few, being distributed over the intercellular space. In the cells of both species viral morphogenesis went along with the accumulation of membranes of rough endoplasmic reticulum and of polysomes in the cytoplasm. On day 4 postinfection the pathological changes were aggravated: edema was developing in the endotheliocytes of the sinusoids and small vessels, congestion was enhanced, and necrosis of Hc was observed. In guinea pigs the blood flow was more severely disturbed, and abundant neutrophils appeared in the zones of virus multiplication in the sinusoids. In monkeys damage to the liver had a diffuse character, whereas in guinea pigs it was focal in nature.

From day 5 to day 7 postinfection increasing numbers of cells were involved in MV reproduction in the liver. Sections of the liver of monkeys contained larger numbers of infected cells and virus particles; more than one half of Hc contained virus-replicative complexes. Virus gemmation occurred on the basal-lateral surface of the cells. Numerous virions were accumulated in the intercellular space and in Disse's space (Fig. 2, *b*). In guinea pigs the majority of infected Hc contained small foci of the viroplasm (Fig. 2, *c*); formation of virions was rarely observed, which indicates that the pattern of MV reproduction in guinea pigs is largely abortive. From day 5 to day 7 the severity of pathological changes in the liver of the two species increased. Necrosis of infected Hc and of individual Hc not involved in virus reproduction was observed. In monkeys the endothelial lining of sinusoids was destroyed, fibrin fibers being observed in their lumen. In guinea pigs the integrity of the sinusoid lining was preserved throughout the course of infection, slight edema being noted only in the endothelium. The absence of signs of inflammatory reaction was characteristic of the liver of monkeys. On the other hand, signs of such a reaction were traced in the foci of virus multiplication in guinea pigs: leukocytes were accumulated in the sinusoids and were released into Disse's space.

MV reproduction was not noted in the spleen of monkeys until day 5 postinfection (Fig. 3, *a*), or in the cells of the MPS of guinea pigs until day 7. In both species the content of virus particles in the sections of the spleen was markedly lower than that in the liver. Infected cells were largely accumulated in the zone of red pulp. Pathological changes, which were more marked in

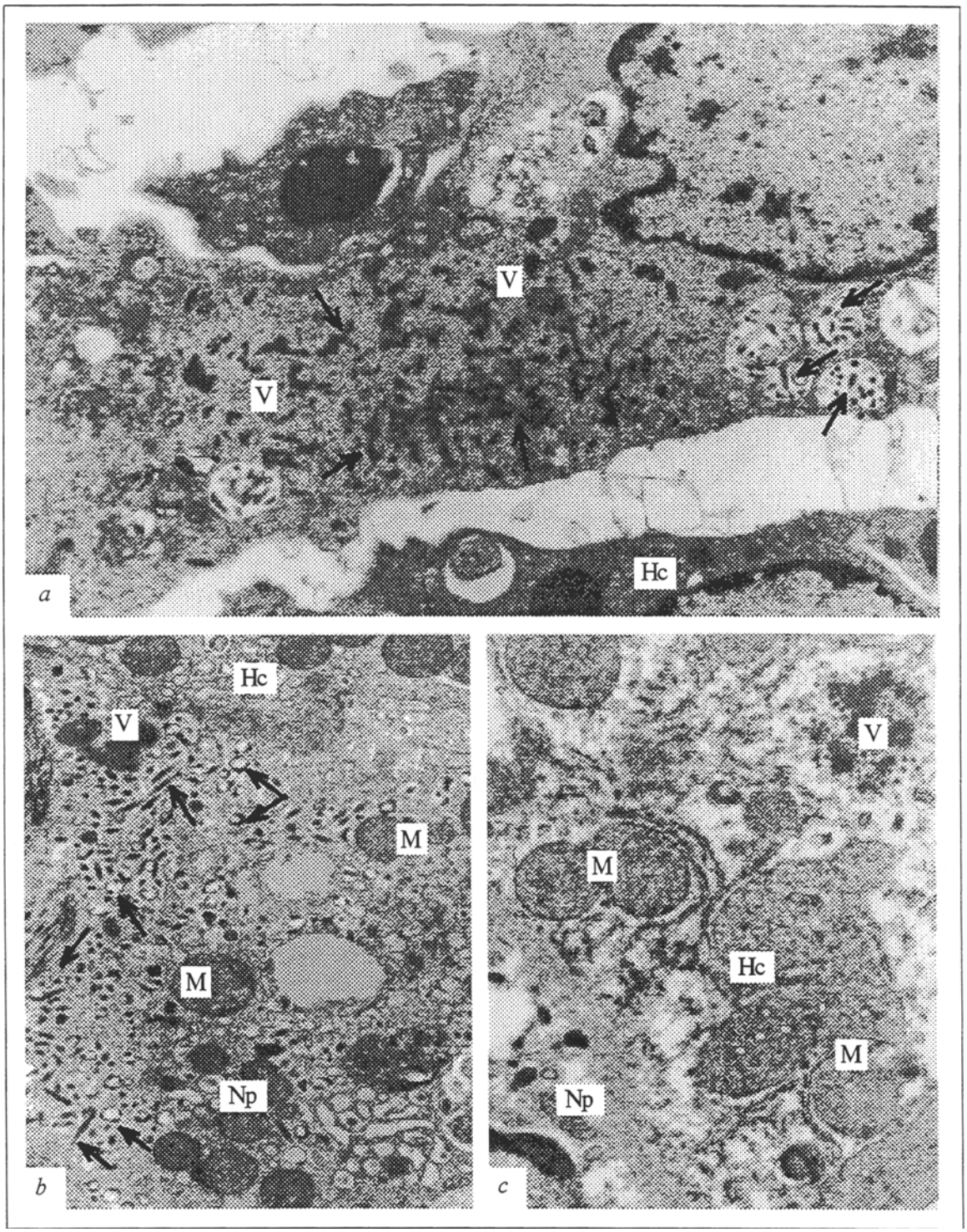

Fig. 2. Reproduction of MV in guinea pig. *a*) MV–infected Kupffer's cell of guinea pig (day 4 postinfection, ×10,000); *b*) virions in grivet liver (6 days postinfection, ×12,000); *c*) infected Hc of guinea pig (5 days postinfection, ×16,000). *Np*: neutrophil; *M*: mitochondria; *V*: viroplasm; *Hc*: hepatocytes. Nucleocapsids and virions marked by one and two arrows, respectively.

monkeys, also developed in this zone. On day 5 blood flow stasis and accumulation of platelets were observed in both species. On days 6-7 the endothelium of sinuses of the red pulp was destroyed, fibrin clots were found in the adjacent tissue, and the formation of thrombi was observed (Fig. 3, *b*). Ruptured blood cells were abundant in the lumen of the vessels. Erythrodiapedesis was observed. In the course of infection numerous erythrophagosome-containing macrophages and large numbers of

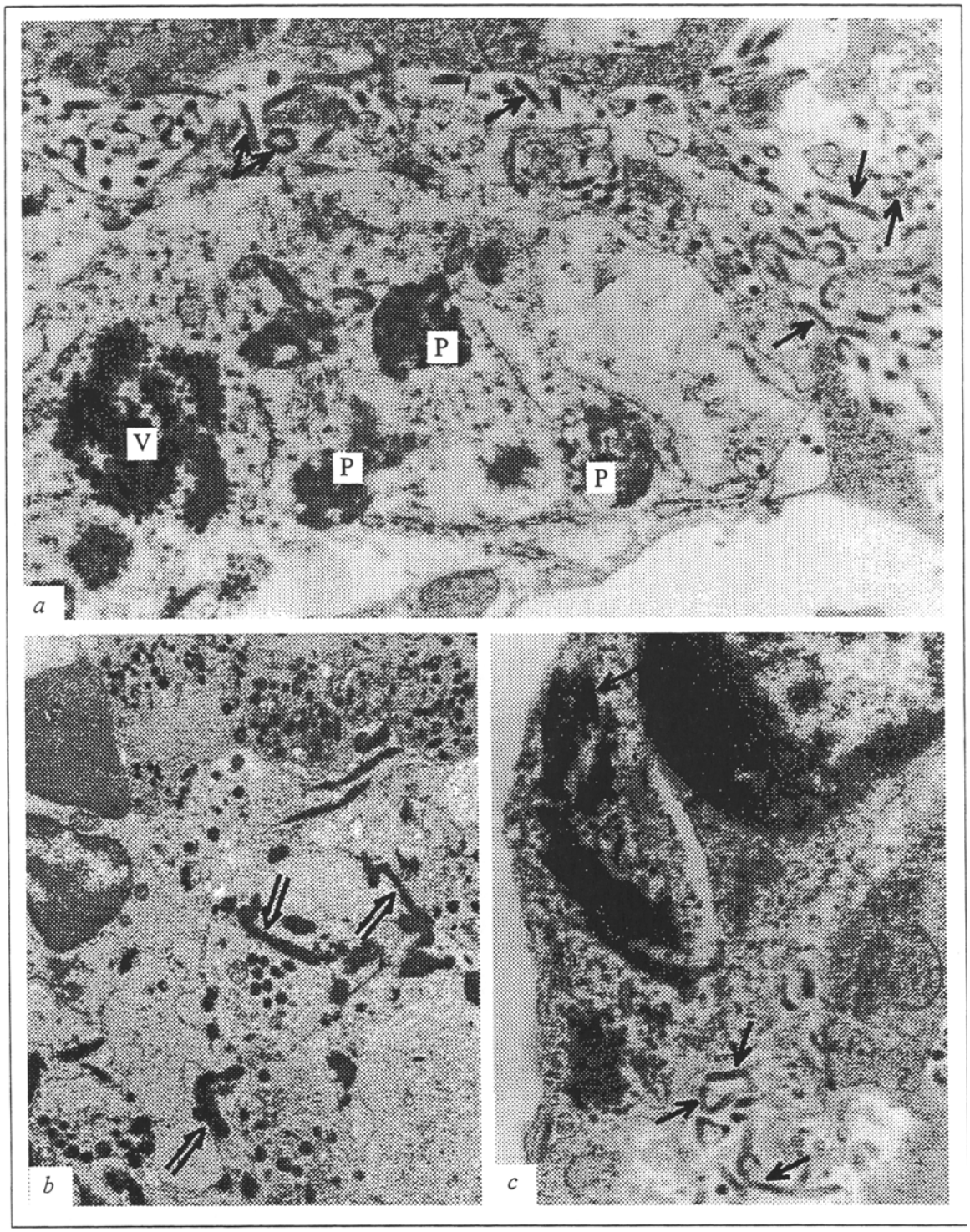

Fig. 3. Reproduction of MV in grivet. *a*) MV–infected MPS cell of grivet spleen (day 5 postinfection, ×16,000); *b*) portion of guinea pig spleen (6 days postinfection, ×8000); *c*) infected endotheliocyte of interalveolar capillary of grivet lungs (6 days postinfection, ×20,000). *P*: phagosome; *V*: viroplasm. Fibrin marked by one arrow, nucleocapsids by two arrows, and virions by double arrow.

neutrophils, which were degranulated and lysed at the final stages of infection, appeared in the red splenic pulp of guinea pigs.

In both species a common feature of damage to the lymphoid tissue of the white splenic pulp and of the mesenteric and inguinal lymph nodes was focal necroses of cells of MPS and of stromal cells, which were detected on days 6-7. In the necrotic zone fibrin precipitation and erythrodiapedesis occurred. About one half of necroses was

due to virus multiplication in the cells of MPS; no morphological signs of infection were observed in other destroyed cells. In contrast to the case with guinea pigs, not only the MPS cells but also the endothelium of postcapillary venules was involved in MV reproduction in the lymph nodes of monkeys. It is worthy of note that lymphocytes were not destroyed in the lymphoid tissue of either species, although in monkeys pronounced depletion of the lymph node cortex and diminution of the white splenic pulp were observed vis-a-vis the initial period of disease. The species-specific differences in the damage to the lymphoid tissue boil down to signs of development of an immune response in guinea pigs and their total absence in monkeys. No mitoses, lymphocyte activation, or plasmacyte formation were found in the monkeys. In guinea pigs foci of multiplication formed on day 4 in the white splenic pulp and in the lymph nodes. Large light lymphoblasts and mitoses in lymphoblasts were seen in the sections, and different stages of plasmacytogenesis were observed. These processes were more pronounced in the lymph nodes than in the white splenic pulp. Plasmacytogenesis in the lymphoid tissue of guinea pigs cannot be regarded as intensive; however, it was rather active, unlike in monkeys, in which signs of plasmacytogenesis were entirely absent. Our findings attest to the blocking of the immune response in the monkeys infected with MV and to its evolution in guinea pigs.

Throughout the course of the experiment pronounced pathological changes were absent in the lungs of both species. Signs of infection were discovered in monkeys on day 6: virions, infected cells of MPS, as well as a few infected endotheliocytes, were encountered in the lumen of capillaries (Fig. 3, *c*). On day 7 infected cells were found in the interstitial tissue. In the lungs of guinea pigs MV multiplication was detected on day 7 postinfection in just solitary MPS cells of the circulatory bed.

In both species changes of the kidneys exhibited a focal pattern and started with the cells of the distal canaliculi, where edema of the cytoplasm and destruction of organelles developed on days 3-4. Later, pathological changes extended to the cells of the proximal canaliculi of neurons, and on days 6-7 they were discovered in the glomeruli, where destruction of podocytes occurred. The majority of glomeruli were degenerated and wrinkled. Signs of inflammation were absent in the renal tissue. In monkeys necrosis of the endothelium of the glomerular and intercanalicular capillaries developed on day 7, the first manifestations being observed from day 4. In guinea pigs changes in the endothelium were less marked, and necroses were absent. MV multiplication in the kidneys of monkeys and guinea pigs proceeded in a few endotheliocytes and in the MPS cells of the circulatory bed; in monkeys infected cells were found on day 7 in the connective tissue.

Our findings demonstrate that in these two species of experimental animals MV multiplies in the cells of MPS, Hc, and, at the final stages of infection, in a few endotheliocytes. The fundamental difference between the two species is the absence of morphological signs of activation of the immune system and of an inflammatory reaction in the monkeys. Our study does not corroborate the conclusion about the crucial role of viral damage to the endothelium in the development of hemostatic disorders in Marburg's hemorrhagic fever, a conclusion which was based on a study of MV reproduction *in vitro* [3]. Analysis of our results attests to the key role of damage to the MPS cells in this process. It is well known that cells of this type mediate the development of hemostatic disorders and of other pathological reactions by releasing an abundance of biologically active compounds [4,5].

REFERENCES

1. M. J. Augler and J. A. Ross, in: *The Macrophage* (Ed. by C. E. Lewis *et al.*), Oxford (1992), pp. 3-56.
2. T. M. Cosgriff, *Rev. Infect. Dis.*, **11**, 5672 (1989).
3. F. A. Murphy, D. I. H. Simpson, S. C. Whitefield, *et al., Lab. Invest.*, **24**, 279 (1971).
4. J. J. Rippey, N. J. Schepers, and J. H. S. Gear, *SA Med. J.*, **66**, 50 (1984).
5. H. J. Snittler, H. Feldman, H. D. Klenk, and D. Drenckhan, *Ann. Anat.*, **174**, 74 (1992).