VIROLOGY

Comparative Study of Pathological Changes in the Mouse Viscera in Infection Caused by Two Orthopoxviruses

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The mechanisms of infection development in intraperitoneal inoculation of mice by ectromelia virus strain K-1 and cowpox strain EP-2 were studied. Ultrastructural parameters of virus assembly and maturation are described. Differences in the types of cells replicating the viruses and in the type of visceral injuries were detected. The studies showed a local type of strain EP-2 cowpox infection and dissemination of ectromelia strain K-1.

Key Words: *orthopoxvirus; mice; electron microscopy; pathogenesis*

Experimental animal models are widely used for evaluating the efficiency of antiviral vaccines and preparations. The most simple approach to evaluation of the drug efficiency is study of its effect on lethal infection of animals (usually mice). Changed mortality level serves as the criterion of drug efficiency [5,13]. Experimental models such as mouse infection with cowpox or mouse pox (ectromelia) are used for evaluating the efficiency of preparations active towards orthopoxviruses (OPV) pathogenic for humans (*Poxviridae* family, including smallpox virus). Ectromelia virus (EV) is pathogenic only for mice and causes a generalized infection eventuating in death even after low-dose infection irrespective of animal age [6,8]. Cowpox virus (CPV) is pathogenic for many mammals, causing, depending on animal species, generalized or local infection, the outcome of which depends on the virus strain, dose, and animal age [1,2]. CPV Strain EP-2 was isolated from an elephant dead during an outbreak of this disease in Nuremberg Zoo [1,10]. The aim of this study is identification of the target cells and comparative evaluation of pathological changes in the mouse viscera during lethal infection caused by EV strain K-1 and CPV strain EP-2.

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MATERIALS AND METHODS

The study was carried out on male outbred albino mice, which were infected by EV strain K-1 in a dose of 50 PFU (plaque-forming units; animals die 6-9 days after infection [8]) and CPV strain EP-2 in doses of 10^6 and 10^7 PFU (animals die 4-6 days after infection [2]). Virus suspension (0.1 ml) was injected intraperitoneally. Strains EV and CPV were cultured in chick embryo chorion allantoic membrane, purified in sucrose density gradient, and titered by the plaque method on a CV-1 cell monolayer [8]. Mice weighing 14-16 g were infected with EV, mice weighing 7-9 g with CPV. Controls were injected with 0.1 ml saline.

The mice were sacrificed by cervical dislocation on days 3-7 after infection daily, 3 animals infected with each strain. Specimens of the liver, spleen, abdominal wall, mesentery were collected, and washings off the peritoneal cavity walls were

made. The samples were fixed in 4% paraformaldehyde. Standard histological processing with embedding the samples in paraffin was carried out for photo-optic examination. The sections were stained with hematoxylin and eosin. Immunohistochemical detection of infected cells on paraffin sections was carried out using human polyclonal antibodies (initial titer 1:10,000) in 1:200 dilution. Viral antigens were visualized using immunoperoxidase-conjugated polyspecific biotinilated antibodies (VIP kits, Vectastain, Vector Lab.). Specimens for ultrastructural study were postfixed in 1% osmium acid, dehydrated routinely, and embedded in epon-araldite. Semithin sections were stained with Azur-2. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Electron microscopic study was carried out on a Hitachi H-600 microscope at ascending voltage of 75 kV.

RESULTS

Ultrastructural parameters of EV K-1 and CPV EP-2 strains replication in mouse cells were similar. The cytoplasm of infected cells contained incorporations consisting of granular substance, immature and mature virions, and sections of immature viral particle membrane fragments (Fig. 1, 2). Morphologically the stages of formation of immature and mature virions of the studied strains did not differ from those described for other OPV [4,11, 12]. The differences between CPV strain EP-2 and EV K-1 manifested at the stage of viral progeny release from the cell. Active release of OPV from the cells was realized by means of the so-called extracellular "coated" virus, one of the main factors of infection dissemination in the body [2,6]. In mouse cells infected with CPV EP-2 strain a coated virus with its extra membrane was rare, while the bulk of daughter virus presented as intracellular "naked" virions. Predominant formation of naked virus is characteristic of replication of other CPV strains as well [8]. Replication of EV strain K-1 in mouse cells led to the formation of numerous extracellular coated viral particles.

Replication of both strains was associated with the formation of virus-specific protein incorporations, directly not related to the virus morphogenesis and maturation. The incorporations were homogeneous, with medium electron density, round. Mouse cells infected with EV K-1 strain contained type A^- incorporations topographically not related to viral particles (Fig. 1, *b-d*). Three variants of incorporations formed in mouse cells during multiplication of CPV strain EP-2: A^- (without viral particles), A^i (with particles located at the periphery of incorporation), and A^+ (with viral particles inside the incorporation) (Fig. 2, *a*, *b*). Replication of EV strain K-1 was paralleled by the formation of one more type of virus-specific structures, directly not involved in the virus morphogenesis: long wavy ribbon formations packed into fibers oriented perpendicularly to the "ribbon" length and divided by gaps of the same length (crystalloid structures).

Ultrastructural parameters of replication of both strains in mouse cells did not differ at the stages of assembly of immature and formation of mature virions. The viruses differed by the number of coated and naked particles and by the morphology of type A incorporations and the presence of crystalloid incorporations.

Electron microscopy made it possible to identify the target cells in which EV K-1 and CPV EP-2 strains replicated in mice and to detect the differences in their "set". In the liver morphological signs of EV K-1 strain virion multiplication were detected in hepatocytes, Kupffer cells, and blood and lymph capillary endotheliocytes. No replication of CPV EP-2 strain was detected in mouse liver. In the spleen EV K-1 strain replicated in macrophages, fibroblasts, endotheliocytes, and poorly differentiated connective tissue cells. CPV EP-2 strain replicated in the same splenic cells, except macrophages. None of the viruses replicated in lymphocytes. The number of cells infected with EV K-1 strain in the spleen was much higher than of cells infected by CPV EP-2 strain, despite a significantly lower infective dose. Reproduction of EV K-1 strain in the mesentery and abdominal wall was detected in macrophages, fibroblasts, myosatellites, endothelial and adventitial cells. The range of target cells for CPV EP-2 strain was wider: the virus replicated in fibroblasts, endotheliocytes, adipocytes, adventitial and smooth-muscle cells (in the abdominal wall also in the striated muscle cells and myosatellites). No replication of CPV EP-2 strain was detected in the mesenteric and abdominal wall macrophages and in peritoneal macrophages washed from the peritoneal cavity walls. EV K-1 strain actively replicated in these cells.

Immunohistochemical study confirmed electron microscopic findings. OPV antigens were detected in hepatocytes and Kupffer cells of mice infected with EV K-1 strain, but no specifically stained cells were detected in the liver of animals infected with CPV EP-2 strain. In the spleen labeled cells were detected in mice infected with both viruses, their location and number differing. After infection by CPV labeled cells were located in the capsule and formed rare small foci in the red pulp. After infection with EV K-1 strain numerous labe-

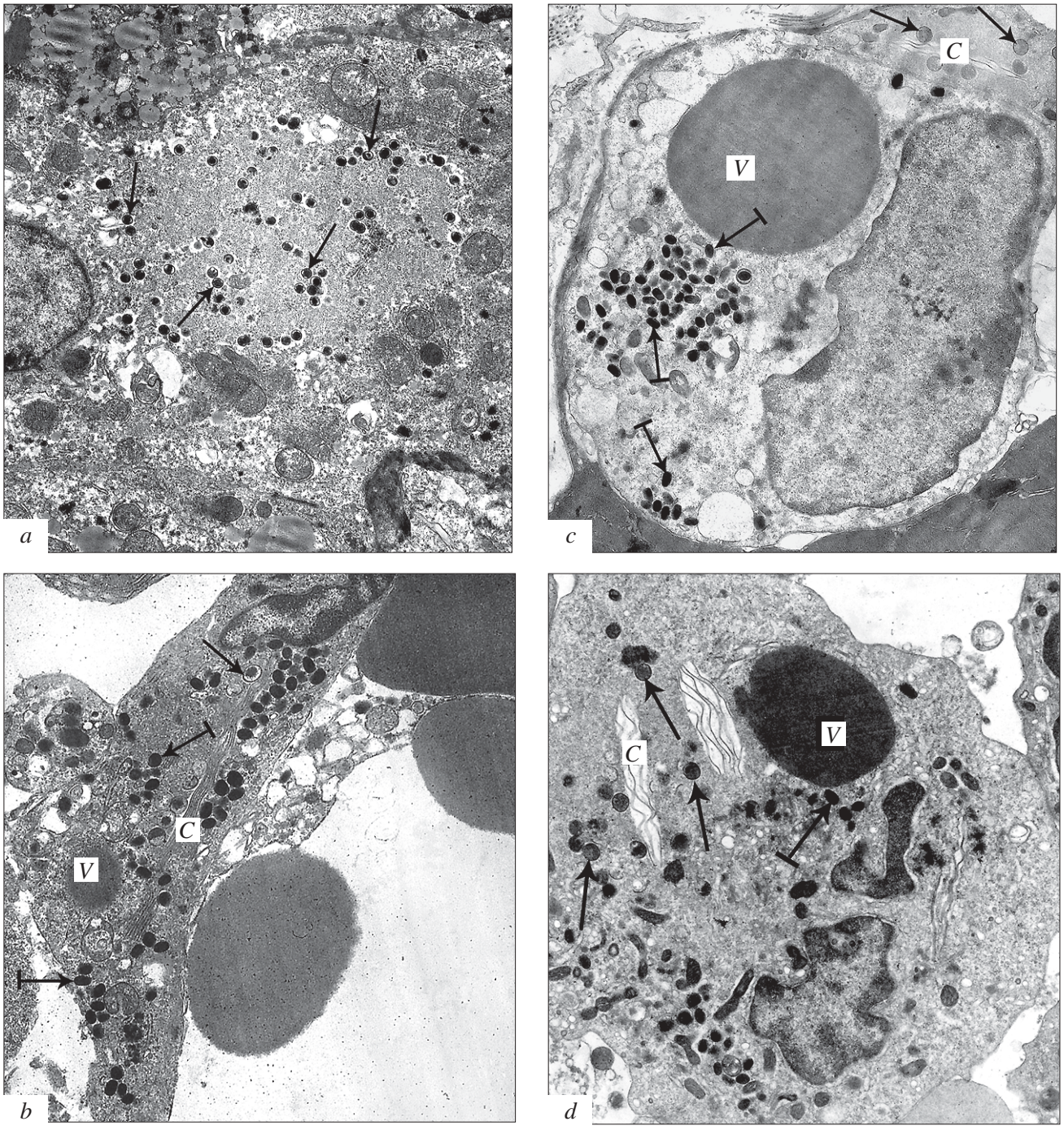

Fig. 1. Replication of ectromelia virus (strain K-1) in cells of mice infected intraperitoneally with 50 PFU (plaque-forming units). *a*) hepatocyte, 6 days postinfection, ×5300; *b*) splenic vascular endotheliocyte, 6 days postinfection, ×9600; *c*) abdominal wall myosatellite, 6 days postinfection, ×9100; *d*) peritoneal macrophage, 4 days postinfection, ×8500. *V*: type A viral incorporations; *C*: crystalloid structures. Arrows show immature virions; underlined arrows show mature virions.

led cells were detected in the red and white pulp of the entire organ stroma.

EV strain K-1 causes generalized infection in mice with pronounced involvement of the liver and spleen [8]. The present findings confirm this observation. Pathological changes in these organs augmented, necroses; inflammatory changes, and circulatory disorders developed during the last days of the disease. In mice infected with CPV strain EP-2 the pathological changes in the liver were the minimum (disorders in the organ circulation). In the spleen CPV strain EP-2 also did not cause appreciable destructive changes, which seemed to be due to a significantly lower level of its replication in

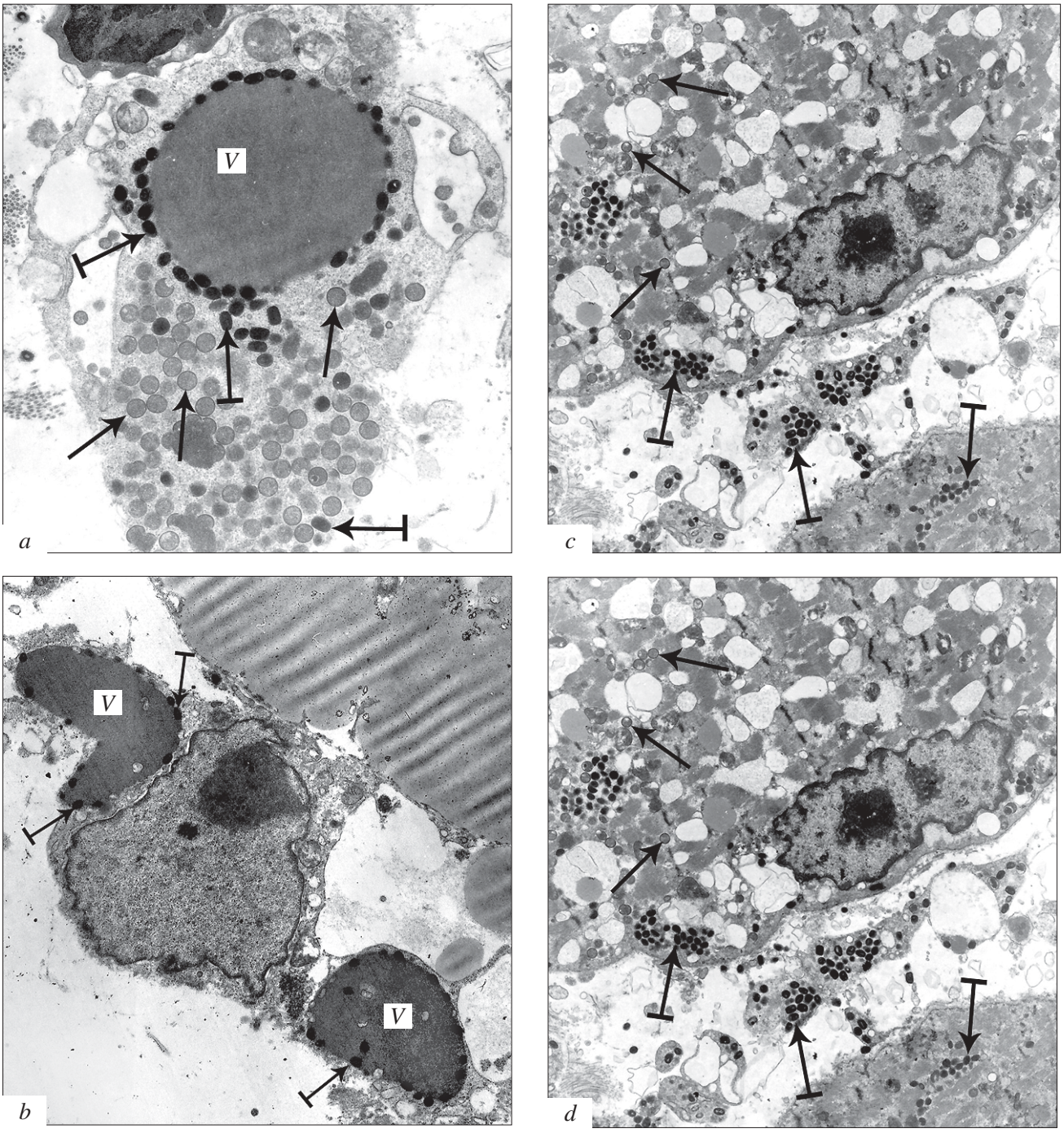

Fig. 2. Replication of cowpox virus (strain EP-2) in the cells of mice infected intraperitoneally. *a*) mesenteric fibroblast, 3 days postinfection (10^7 PFU), ×1100; *b*) mesenteric adipocyte, 4 days postinfection (10^7 PFU), ×7100; *c*) abdominal wall muscle cells and fibroblast, 6 days postinfection (10^5 PFU), ×5800. *V*: type A viral incorporations. Arrows show immature virions; underlined arrows show mature virions.

comparison with EV strain K-1. Lymphocyte and neutrophil apoptosis in the white pulp follicles was observed in the spleen of mice infected with both strains (Fig. 3), while the nuclei of infected cells of all types retained their normal structure.

Significant differences in the type of injuries were seen in tissues adjacent to the site of virus injection into the peritoneal cavity. Rare small foci were seen in the mesentery and abdominal wall of mice injected with EV strain K-1. These foci contained infected cells, small hemorrhages under the mesothelial basal membrane, small depositions of fibrin in connective tissue, and few neutrophils. The mesothelium remained intact. The degree of

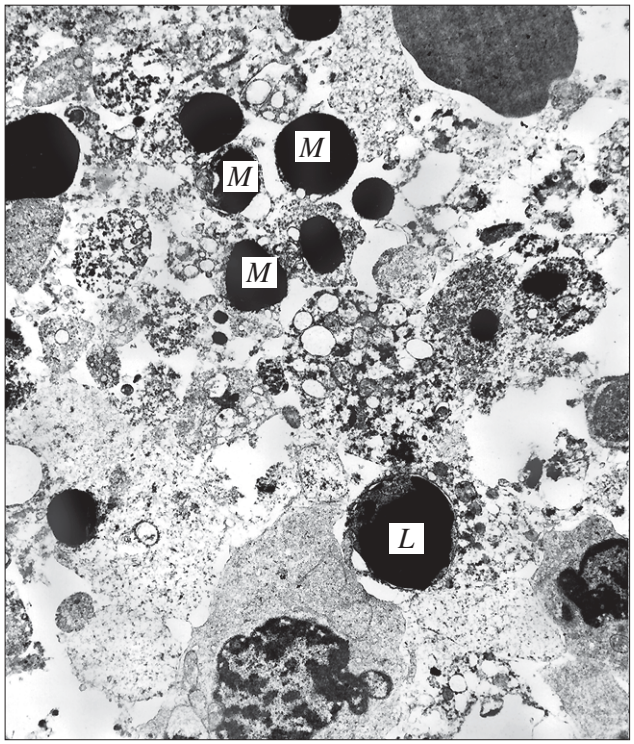

Fig. 3. Lymphocyte apoptosis and destroyed splenic macrophage in mouse infected intraperitoneally by 50 PFU of ectromelia virus (strain K-1), ×5500. *L*: lymphocyte; *M*: fragments of macrophage cytoplasm and phagosomes.

damage to these tissues was much more pronounced in mice infected with CPV EP-2 strain. The mesothelium was desquamated, the peritoneal cavity was limited by the basal membrane. Foci of lesions were more numerous, they were significantly larger, with massive hemorrhages and fibrin depositions, numerous necrotic and infected cells.

Analysis of the findings indicates that EV K-1 and CPV EP-2 strains, belonging to related *Orthopoxvirus* species, cause infections of different types in experimental animals of the same species. EV strain K-1 leads to a systemic disease with the involvement of the liver and spleen, while CPV strain EP-2 causes local infection, not involving the liver. Replication of CPV EP-2 strain is confined to tissues adjacent to the site of the virus application; massive replication of the virus in these tissues causes peritonitis. We found that CPV EP-2 strain was incapable of replicating in macrophages, which was not typical in the presence of productive infection of other cells, including well-differentiated ones. Presumably, the inability of CPV strain EP-2 to replicate in macrophages is responsible for the failure of virus dissemination and just local infection. This is in line with previous data on the role of macrophages in the development of CPV infection [8,9]. Hence, a lethal viral infection can develop differently in the same animal species, which should be taken into consideration when evaluating the efficiency of antiviral preparations.

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