PRIMATOLOGY

Encephalomyocarditis in Monkeys

R. I. Krylova and E. K. Dzhikidze

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 3, pp. 337-341, March, 2005
Original article submitted December 23, 2003

This paper presents the results of studies of a spontaneous viral infection in monkeys -- encephalomyocarditis caused by encephalomyocarditis virus. The infection first detected in the Sukhumi Breeding Center in 1974 was observed in the Adler Breeding Center since 2001. The characteristics of the virus are described and principles of diagnostic by the results of pathologic studies are presented.

Key Words: *encephalomyocarditis; monkeys of different species*

Encephalomyocarditis (EMC) virus strains were isolated in different geographic zones from humans and many wild and domestic animals. The incidence of virus-neutralizing antibodies to EMC virus reaches 34% in children and 50% in adults in different countries. These viruses cause a variety of diseases in humans; sometimes it is just mild fever without definite symptoms, in other cases fever is associated with neurological disorders, up to paralytic forms with lethal outcomes. Clinical manifestations of the infection in animals also greatly vary. Epizooties in swine, particularly in young animals, kill 30-80% animals at the farms; the disease can also occur in cattle and horses. Natural hosts of the virus are wild rodents.

Monkeys are highly sensitive to the virus; numerous EMC strains were isolated from these animals. The first strain was isolated in Florida zoo from a dead chimpanzee with a picture of acute almost total myocarditis. Similar changes were previously detected in a dead gibbon. The infection in this zoo was observed over many years in different animals; 24 virus strains were isolated from 8 animal species, including the strains from monkeys (baboons, gibbon, chimpanzee). All animals died suddenly with a picture of acute necrotic myocarditis. Myocarditis caused by EMC virus is known for rhesus monkeys, orang-outangs [4], baboons, green monkeys [5], and Southern-American monkeys (night and squirrel monkeys) [5]; in these latter ones the infection was characterized by development of myocarditis and encephalitis. The infection can be maintained for a long time in zoos, most often among monkeys. The infection can lead to high mortality among monkeys in primatological centers, as it happened in the colony of baboons in San-Antonio, where 80 of 3060 animals died from EMC during 9 months [3,6].

EMC virus is detected everywhere, it is widely used for modeling viral cardiac disease and for studies of the etiological role in the pathogenesis of diabetes mellitus [2,7], but the data on this virus in Russian literature are scanty. On the other hand, strains of this virus are prevalent at least at the territory near the Black Sea, which fact we traced in our study of diseases of monkeys in the Sukhumi and Adler Breeding Centers. The first cases of infection were recorded in 2 rhesus monkeys which died in 1970. EMC-70 strain was isolated from these monkeys. Excretions from rodents (mice, rats) living in Sukhumi Center, were the source of infection. Six rhesus monkeys and 15 Papio hamadrias died in the Center in 1982-1988. Animal deaths were recorded under semi-natural conditions at the Zapadnaya Gumista sanctuary for baboons, where 4 Papio hamadrias died in 1978-1979. EMC was recorded in the Adler Breeding Center. Fourteen monkeys died from EMC from November, 2001, to

Institute of Medical Primatology, Russian Academy of Medical Sciences, Sochi. ***Address for correspondence:*** iprim@sochi.net. R. I. Krylova

0007-4888/05/1393-0355

September, 2003: 2 *Macaca mulatta*, 3 *Cercopithecus aethiops*, and 9 *Papio hamadryas*. Virus strains isolated from dead monkeys were identical in all cases and their characteristics were intrinsic of EMC virus (RNA genome virus, *Picornaviridae* family). This report presents the description of spontaneous infection of monkeys and its diagnosis.

MATERIALS AND METHODS

Autopsy material from 42 dead monkeys was examined (10 *Macaca mulatta*, 3 *Cercopithecus aethiops*, and 29 *Papio hamadryas*), 14 of these monkeys from the Adler center and 28 from the Sukhumi center.

A 10% suspension was prepared from monkey organs and tissues for virological study, and cell cultures MA-104, Vero, FL, L, RH, and SPEV were infected with this suspension. The cells were cultured routinely in medium 199 with 10% cattle serum. The major cultural studies, such as virus titration with evaluation of TCD_{50}, were carried out on MA-104 culture.

Studies of the sensitivity of small laboratory animals to the virus were carried out on random-bred albino mice of different weight, guinea pigs (200-250 g), and rabbits (newborn and young). Virus-containing material (10% organ suspension or culture fluid) was injected into the brain (0.02 ml), subcutaneously (0.05 ml), intramuscularly (0.3 ml), or by combined method.

The characteristics of infection in monkeys were studied in 25 monkeys of 4 species (13 *Macaca mulatta*, 5 *Cercopithecus aethiops*, 3 red monkeys, and 4 *Papio hamadryas*). The monkeys were infected intraperitoneally in the majority of cases; in some cases the material (1.2-1.5 ml culture fluid of MA-104 cell culture with $TCD_{50/ml}$ titer $10^{8.5}$) was injected into the brain, subcutaneously, or given orally.

In serological studies the sera from rabbits and guinea pigs immunized with the appropriate viruses served as immune sera to the isolated EMC strains (including the primary isolated EMC-70 strain and the prototype EMC strain from Pasteur Institute, Paris, which was maintained by passages in L cell culture). The prototype strains of poliomyelitis virus (types I-III), Coxsackie (group B, 6 types, and group A, 23 types), ECHO (16 types), and standard antisera to them were received from WHO. Virus neutralization test was carried out routinely in MA-104 and L cell cultures. Before HAI test, the sera were treated in acetone, incubated with 4 hemagglutinating units of antigen during 18 h at 4°C, after which 0.5% suspension of sheep erythrocytes was added.

The material for pathological studies was fixed in 10% neutral formalin. Paraffin sections of the viscera, striated muscles, and fatty tissue were stained with hematoxylin and eosin; cerebral and spinal sections were stained with thionine by Niessle's method.

RESULTS

The disease of monkeys was characterized by a seasonal pattern: the greatest number of cases was recorded during autumn-winter and early spring. Sporadic cases and episodes of group deaths of monkeys were recorded during several years in the same cages, the males predominating (27 of 42).

Macroscopic examination showed fluid in the pleural and pericardial cavities, edema and sharp hyperemia of the lungs in all dead monkeys. Hypertrophy and fatty tissue edema in the axillary region and moderate hyperplasia of axillary lymph nodes were found in the majority of monkeys. Whitish areas were detected in the hyperemic hearts of some animals. Histological study showed myocardial involvement in monkeys of all species, presenting as large focal or diffuse myocarditis with death of muscle fibers and massive lymphohistiocytic (sometimes with admixture of an appreciable number of segmented leukocytes) infiltration in foci of cardiomyocyte death and in the interstitial tissue (Fig. 1, *a*). In some cases infiltration involved the epicardium. Fiber fragmentation, sarcoplasma lysis, and predominantly lymphohistiocytic infiltration were seen in the striated muscles of the hips, neck, and upper limbs (Fig. 1, *b*). Inflammation in the fatty tissue of the axillary region and interscapular area was characterized by damage lipocytes with multifocal accumulation of fat. Lymphocytes predominated among infiltration elements in foci of lipocyte death (Fig. 1, *c*). Changes in CNS, most pronounced in baboons and less incident in rhesus and green monkeys, were characterized by cerebral edema, loosening of vascular walls, hyperemia, capillary stasis, and presence of glial nodules and perivascular lymphohistiocytic infiltration (Fig. 1, *d*). Neuronal involvement presenting as swelling of bodies and processes, perinuclear chromatolysis, cytoplasm vacuolation, nuclear lysis, and rare neuronophagia, was seen in the brain stem and cerebellar nuclei. No appreciable loss of neurons was observed.

The virus was isolated after infection of albino mice, guinea pigs, and cell cultures by 10% suspension of dead monkeys' organs. Cytopathogenic changes were seen in all cell cultures. Titration of organ suspension in MA-104 cell culture showed dissemination of the virus in experimental animals, including monkeys. The virus was first detected in the blood, brown fat, heart, brain cells, then appeared in the spleen, lymph nodes, lungs, and liver. The virus was also present in rectal washings and nasopharyngeal mucus of monkeys, which seemed to be epidemiologically

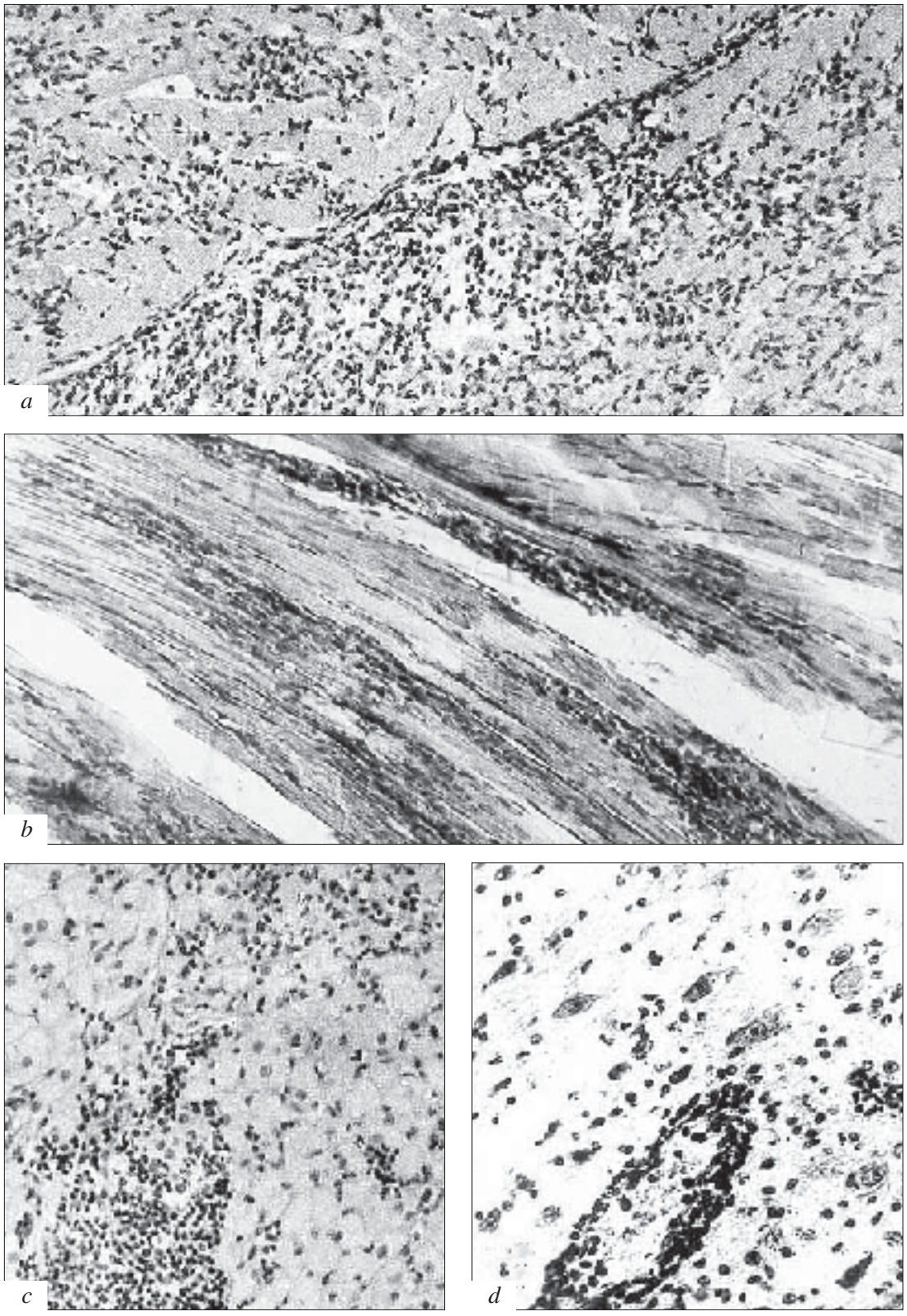

Fig. 1. Organ involvement in monkeys with encephalomyocarditis. *a*) parenchymatous interstitial myocarditis in a green monkeys, ×80; *b*) myositis of the neck muscles in a baboon, ×60; *c*) brown fat involvement in a rhesus monkey, ×80; *d*) vasculitis, glial activation, perinuclear chromatolysis in the medulla oblongata neurons of a rhesus monkey, ×120; *a-c*) hematoxylin and eosin staining; *d*) thionin staining after Niessle.

significant for dissemination of the infection in the group of monkeys.

The virus exhibited hemagglutinating activity towards human erythrocytes (0 group), sheep, guinea pig, but not rhesus monkeys erythrocytes.

The virus possessed a high spectrum of pathogeneity for animals; 100% suckling albino mice died 24-72 h after inoculation without apparent clinical signs of disease. Adolescent mice developed a disease with paralysis of the hind and sometimes fore limbs. More than half of animals died on days 3-5. Hind limb paralysis and death on days 4-6 after infection were observed in half of adult mice. Pathomorphological study revealed changes in the striated muscles (myositis) and severe cell degeneration in the brown fat, combined with massive lympholeukocytic infiltration. One-third of mice developed myocarditis, half of adult mice encephalitis and meningoencephalomyelitis. The titers of the virus were the highest in mouse brain, spleen, and liver.

More than one-third of infected guinea pigs fell ill (fever, adynamia, leukopenia, hind limb palsy). Macroscopic examination showed whitish areas in the myocardium, hyperemia, meningeal and cerebral tissue edema in dead guinea pigs. Histological study showed diffuse (mainly interstitial) myocarditis with massive lymphohistiocytic infiltration of the intermuscular and perivascular spaces. Mild encephalitis with involvement of the brain stem and cerebellum was detected in almost all guinea pigs. The virus titers were maximum in the brain, heart, and liver.

Adolescent rabbits were little sensitive to the virus; about 10% newborn rabbits fell ill and died with sharp adynamia and hind limb paralysis. The main pathomorphological changes were diffuse myocarditis and encephalitis, rarely changes in the brown fat and striated muscles.

In rhesus monkeys the sensitivity to infection varied from inapparent infection to a severe lethal disease (in 4 of 13 monkeys). Severe disease with lethal outcome was observed in 3 of 5 infected green monkeys and in 2 of 3 red monkeys. Two of the four infected baboons fell ill, one of these died and the other survived the acute period and was sacrificed 2.5 months after infection. The main clinical signs of disease were sharp weakness, anorexia, tachycardia, and ECG changes (signs of myocarditis or large focal myocardial infarction). Pathomorphological study showed inflammatory changes of different intensity in the brown fat and striated muscles, myocarditis, and involvement of the nervous system (slight encephalitis). The changes in the myocardium were always significant and presented as a severe parenchymatous interstitial inflammation. Morphological analysis in the baboon, which survived the acute period and was sacrificed 2.5 months after infection, showed a large focal sclerosis of the cardiac muscle, similar to the postinfarction cicatrix.

Serological identification of the virus in the HAI test with antisera to picornaviruses, including the prototype EMC virus, showed positive result only with the antiserum to the prototype virus. The titer of hemagglutinating antibodies was 1:1024-1:2048, *vs.* below 1:8 in the rest sera. Antigenic relation of the virus strains, studied in the neutralization test in cell culture with standard antisera to Coxsackie A and B viruses, confirmed the absence of antigenic relation to these viruses (antisera diluted 1:10-1:100 did not neutralize 1-10 virus doses). The titers of neutralizing antibodies in cross neutralization tests in cell culture with 100 doses of virus strains with antisera to the prototype (EMC) and the studied virus strains were 1:512-1:1024.

Hence, studies of the characteristics of the isolated virus strains and the presence of antigenic similarity with the prototype EMC strain indicate that the isolated strains belong to the EMC group viruses. The type of myocarditis and encephalitis did not differ from those in spontaneous infection of monkeys caused by these viruses [6] and in experimental infection [2].

The strains caused significant inflammatory changes in the brown fat and striated muscles not only in spontaneous and experimental infection of monkeys, but in laboratory albino mice, which is considered pathognomonic for infection with Coxsackie viruses (mainly group B). We found that EMC group viruses are characterized by the same effect; it means that Coxsackie infection cannot be diagnosed on the basis of the pathomorphological findings alone. We should not rule out the possible role of EMC virus (not only Coxsackie viruses) in the etiology of viral myocarditis or as a cause of sudden death of animals and humans. High prevalence of the virus, including at the territory of our country, does not permit us rule out its role in the etiology of some cases of myocardial infarction, diagnosed by ECG findings. This probability is particularly high for young patients, in whom no morphologically changed coronary vessels or signs of their thrombosis are found and who have no history of clinical signs of coronary insufficiency.

The diagnosis of EMC in monkeys in acute cases is based on the signs of involvement of the myocardium, nervous system, and particularly striated muscles and brown fat. Remote diagnosis is much more difficult, when monkeys die from heart failure in the presence of cardiosclerosis, including large focal one. A history of EMC infection seems to be the most probable cause of cardiosclerosis in monkeys without atherosclerotic changes in the vessels. Correct diagnosis of the etiology of myocarditis should be based on not only bacteriological, but also virological findings.

REFERENCES

1. E. K. Dzhikidze, R. I. Krylova, E. Ya. Balaeva, *et al.*, *Vopr. Virusol.*, No. 4, 34-38 (1982).
2. J. L. Blanchard, K. F. Soike, and G. B. Baskin, *Lab. Anim. Sci.*, **37**, 635-639 (1987).
3. K. D. Carey, G. B. Hubbard, K. F. Soike, *et al.*, *Ibid.*, **38**, 493 (1988).
4. S. B. Citino, B. L. Homer, G. M. Gaskin, *et al.*, *J. Zoo Anim. Med.*, **37**, 214-218 (1988).
5. J. M. Gaskin, M. A. Jorge, C. F. Simpson, *et al.*, *Ann. Proc. Am. Ass. Zoo Vet.*, 1-70 (1980).
6. G. B. Hubbard, K. F. Soike, T. M. Batler, *et al.*, *Lab. Anim. Sci.*, **42**, 233-239 (1992).
7. J. W. Yoon, W. Ko, Y. S. Bae, *et al.*, *J. Gen. Virol.*, **69**, 1085-1090 (1988).