— Note —

Susceptibility of Four Species of Small Rodents to Encephalomyocarditis (EMC) Virus Infection

Hitoshi MATSUZAKI, Kunio DOI, Chiyo DOI, Takashi ONODERA*, and Tomotari MITSUOKA

*Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Bunkyo 113, Japan and *Laboratory of Immune Cytology, National Institute of Animal Health, Kannondai 3-1-1, Tsukuba-shi, 305*

(Received 10 May 1989/Accepted 1 July 1989)

The D variant of encephalomyocarditis virus (10^1 – 10^5 PFU/head) was intra-peritoneally inoculated into 4 species of small rodents, rats, mice, Syrian hamsters, and Mongolian gerbils, and the susceptibility of these animals to EMC virus was examined virologically and histopathologically 3 days after infection. Viral replication was detected in the brain (mice), in the heart (mice and gerbils), and in the pancreas (mice, hamsters, and gerbils). No viral replication was detected in rats. Histopathological changes were seen in the brain (mice and hamsters), in the heart (mice and gerbils), and in the pancreas (mice, hamsters, and gerbils). No histopathological changes were seen in rats. The present results suggest that it may be quite possible to produce EMC virus-induced diabetes mellitus not only in mice but also in hamsters and gerbils.

Encephalomyocarditis (EMC) virus has been attracting great attention as a useful tool for inducing a diabetes mellitus (DM)-like syndrome in certain strains of mice [4]. Although it was recently reported that mice infected with EMC virus were also useful for studying the complications of DM when a suitable combination of a variant of EMC virus and a particular strain of mice were employed [7], rodents larger than mice would be more convenient for the investigation of complications of DM from a technical standpoint. On the other hand, EMC virus is an important causative agent of spontaneous myocarditis in pigs [1], and rodents, especially rats, are considered to be reservoir hosts or carriers of EMC virus infection in pigs. Reports of rodent susceptibility to EMC virus infection are few however except in the case of mice [3, 5]. This paper describes the systemic virology and histopathology of 4 species of rodents, i. e., mice, rats, Syrian hamsters and Mongolian gerbils (*Meriones unguiculatus*), infected with EMC virus.

The D variant of EMC virus (EMC-D) [6] cultured on mouse L 929 cells (titer, 3.5×10^6

PFU/ml) was diluted with 0.01 M PBS (10^1 , 10^3 and 10^5 PFU/head) and inoculated i. p. into 3 or 4 animals per dilution (Table 1). Animals given PBS alone served as controls. Animals were killed by exsanguination under ether anesthesia 3 days after inoculation. The brain, heart, lungs, liver, spleen, pancreas and kidneys were removed from each animal and divided into two parts. One was subjected to viral titration by plaque assay on L 929 cells [2], and the other was fixed in 10% neutral buffered formalin for histopathological examination. Four μ m-paraffin sections were made and stained with hematoxylin and eosin (HE). Blood samples collected from these animals were also subjected to viral titration.

As shown in Table 1, deaths occurred only in gerbils, and mortality was higher in females than in males. At necropsy, marked increase in volume due to serous ascites and prominent subcapsular and interlobular edema of the pancreas were noted in the Syrian hamsters. TUM gerbils exhibited the same gross changes as the Syrian hamsters, while JMS gerbils did not. Pancreatic swelling was also observed in mice. White patchy lesions were found in the

Table 1. Animal mortality 3 days after inoculation with EMC-D

Virus titer (PFU/head)	10 ¹	10 ³	10 ⁵
Mice			
DBA/2 m	0/4	0/4	0/4
DBA/2 f	0/4	0/4	0/4
BALB/c m	0/4	0/4	0/4
BALB/c f	0/4	0/4	0/4
Rats			
SD m	0/3	0/3	0/3
Wistar m	0/3	0/3	0/3
Syrian hamsters			
APA m	0/3	0/3	0/3
APA f	0/3	0/3	0/3
Golden m	0/3	0/3	0/3
Gerbils			
TUM m	0/3	1/3	3/3
TUM f	3/3	3/3	3/3
JMS m	1/3	1/3	N.D.
JMS f	1/3	3/3	N.D.

Values are number of dead/number in group

N.D.=Not done m: male f: female

right ventricular epicardium of a small number of gerbils and of almost all of the mice. Similar but less severe epicardial lesions of a spontaneous nature were found in mice. Rats had no gross lesions.

In Syrian hamsters, viral replication was detected only in the pancreas (Fig. 1 a). In mice, virus titers higher than in the blood were recorded in the heart and pancreas of the DBA/2 strain and in the brain, heart and pancreas of the BALB/c strain. The virus titer in the pancreas of mice was significantly higher in males than in females (Fig. 1 b). In gerbils, viral replication was detected in the pancreas of the TUM strain and in the heart of both strains (Fig. 1 c). On the other hand, no viral replication was demonstrated in any of the organs of the rats examined.

Brain lesions characterized by focal degeneration and pyknosis of neurons in the pyramidal layer of the hippocampus were found in a small number of BALB/c mice and APA hamsters (Fig. 2). Scattered myocardial necrosis with calcification or inflammatory cell infiltration was common to mice and gerbils, but its severity was generally higher in gerbils than in mice (Fig. 3). The pancreas was more or less involved by lesions in all species except rats. Mice and Syrian hamsters

Table 2. Distribution and incidence of histopathologic lesions

dose	Pancreas								
	Heart			acinar cells			islet cells		
	10 ¹	10 ³	10 ⁵	10 ¹	10 ³	10 ⁵	10 ¹	10 ³	10 ⁵
Mice									
BALB/c m	+	+	+	+	±	±	±	±	±
BALB/c f	+	+	+	+	+	+	±	±	+
DBA/2 m	+	+	+	—	±	±	—	±	±
DBA/2 f	+	+	+	+	+	±	±	+	+
Syrian hamsters									
APA m	—	—	—	±	±	±	—	±	+
APA f	—	—	—	±	±	±	—	+	±
Golden m	—	—	—	±	±	±	—	—	+
Gerbils									
TUM m	±	+	±	+	±	±	—	±	±
TUM f	+	+	±	+	±	±	—	—	—
JMS m	+	+	N	±	±	N	±	±	N
JMS f	±	N	N	±	N	N	±	N	N

N: Not done —: No lesions ±: Rare and mild lesions +: M lesions

±: Moderate lesions #: Severe lesions

Fig.1. Viral replication in organs 3 days after inoculation with EMC-D m: male f: female
 *: Only one sample was examined, ☆: Not examined ★: Viral replication was not observed Br: brain H: heart Lu: lungs Li: liver Sp: spleen Pa: pancreas Ki: kidneys Bl: blood

had lesions both in the islets of Langerhans and in the exocrine glands, viz, massive necrosis of islet cells and focal necrosis of acinar cells with slight to moderate interstitial inflammatory exudation were present in mice of both strains (Fig. 4 a). In comparison, islet cells were affected only focally and slightly in some animals, while exocrine glands were massively damaged by necrosis of acinar cells and interstitial inflammatory exudation in Syrian hamsters (Fig. 4 b). Clear strain differences in the severity of pancreatic lesions were detected in gerbils. JMS gerbils exhibited only slight focal acinar cell necrosis, whereas TUM gerbils showed massive necrosis of acinar cells with marked inflammatory exudation (Fig. 4 c). The islets of Langerhans of the TUM gerbils were rarely involved with inflammation. Rats had no histopathological changes in any of the organs examined. The incidence and distribution of lesions are shown in Table 2.

As mentioned above, overall susceptibility to EMC virus infection varied among the 4 species of rodents, and except for mice, only Syrian hamsters showed slight islet of Langerhans cell lesions in the acute stage of EMC virus infection. These results indicate that Syrian hamsters, in addition to mice, may be quite likely to develop DM on the basis of further examinations of the dose of virus inoculated and the age of the host. As far as gerbils are concerned, using EMC virus adapted to the gerbil's islet cells may be effective in inducing DM. Recently we have succeeded in adapting EMC-D to *in vitro* cultures of gerbil islet cells in our laboratory, and infection of this islet cell-adapted virus in gerbils will be carried out before long.

Contrary to our expectations, infection was not established in rats inoculated with EMC-D in the present study. In this connection, further investigations using variants of EMC virus other than EMC-D will be needed.

The authors wish to express gratitude to Dr. K. Yoshihara, National Institute of Animal Health, for technical assistance in the virological experiments.

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4種の小型げっ歯類の脳心筋炎ウイルスに対する感受性

松崎仁志・土井邦雄・土居千代
小野寺 節*・光岡知足

東京大学農学部実験動物学教室
*農林水産省家畜衛生試験場免疫細胞研究室

4種の小型げっ歯類の脳心筋炎ウイルスD変異株に対する感受性を調べ、糖尿病発症の可能性について検討した。ラット、マウス、シリアンハムスター、スナネズミに、 10^1 , 10^3 , 10^5 PFUのウイルスを腹腔内接種し、3日後に剖検して臓器内ウイルス力価の測定及び組織学的検索を行なった。ウイルスの分離は、マウスの脳、心臓、脾臓、シリアンハムスターの脾臓、スナネズミの心

臓及び脾臓で確認された。組織病変は、マウスの脳、心臓、脾臓、シリアンハムスターの脳、脾臓、スナネズミの心臓と脾臓で認められた。ラットではウイルスの分離、組織病変ともに確認できなかった。以上から、マウス以外にもシリアンハムスターやスナネズミで糖尿病誘発の可能性が示唆された。

Explanation of Figures

- Fig. 2. Cerebrum of a male APA hamster 3 days after inoculation with 10^5 PFU of EMC-D. Degeneration of neurons in the pyramidal layer of the hippocampus (arrow) HE $\times 200$
- Fig. 3. Heart lesions of a female DBA/2 mouse 3 days after inoculation with 10^5 PEU of EMC-D. Myocardial cell necrosis in left ventricular wall HE $\times 75$
- Fig. 4a. Pancreas of a male DBA/2 mouse 3 days after inoculation with 10^3 PFU of EMC-D. Marked islet cell necrosis with inflammatory edema and necrosis of acinar cells HE $\times 200$
- Fig. 4b. Pancreas of a male Golden hamster 3 days after inoculation with 10^5 PFU of EMC-D. Acinar cell necrosis with inflammatory edema. The structure of small numbers of islets has been affected HE $\times 200$
- Fig. 4c. Pancreas of a male TUM gerbil 3 days after inoculation with 10^4 PFU of EMC-D. The structure of the islet cells is almost normal. HE $\times 200$

Fig. 2	Fig. 3
Fig. 4 a	Fig. 4 b
Fig. 4 c	