REPRODUCTIVE DISORDERS IN FEMALE SHR RATS INFECTED WITH SIALODACRYOADENITIS VIRUS

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ABSTRACT

Effects of sialodacryoadenitis virus infection on the reproduction of female SHR rats were studied. The oestrous cycle was considerably perturbed in most infected rats, the perturbation was observed initially between Days 0 to 10 post infection and the effect persisted for 6 to 18 days. About half of the foetuses of dams infected on Day 0 of gestation were found dead while only 4% of the foetuses from non-infected dams were found dead. Five or six days after infection on Day 0 of gestation, some infected dams were shown to have metritis, and virus antigen was detectable within the endometrium as well as exudate cells. In dams infected on Day 5 or later of gestation and severely diseased, the offspring showed a low survival rate possibly because of inadequate nursing.

INTRODUCTION

In pregnant mice infected with mouse hepatitis virus (MHV) foetal death due to vertical transmission of the virus was observed (1,2,3). We previously reported that breeder rats naturally infected with sialodacryoadenitis virus (SDAV), another member of coronaviridae, showed apparent disorders in the oestrous cycle and lowered reproduction rate (4). The experiments described below were designed to effects of experimental SDAV infection on the oestrous cycle as well as gestation of female SHR rats; this strain of rat is known to be highly susceptible to the infection (4).

MATERIALS AND METHODS

Animals. Male and female SHR rats, F14 of brother-sister mating, were supplied in 1968 by the Department of Pathology, Kyoto University Medical School. They were randomly bred at this laboratory, and a Caesarean-derived colony was established in 1972 (5). The colony had been serologically monitored for

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freedom from SDAV infection (6). Animals were kept on a commercial bedding (White Flake, Charles River Japan, Atsugi) in metal cages (200x305x130mm) and given pellets(CA-1, Japan CLEA, Tokyo) and tap water. The animal room was kept at 24±2°C, 55±5% relative humidity and lighting for 12 hours per day. The air was exchanged ten times/hour. Animals were used for experiments at 13 to 19 weeks of age. Each morning Giemsa-stained vaginal smear preparations were examined microscopically and females found to be at the pro-oestrus were mated.

SDAV. Strain TG of SDAV (7) from a naturally infected rat was subjected to seven passages in rats by intranasal (i.n.) inoculation of infected sub-maxillary gland tissue. The affected gland tissue was homogenized in phosphate buffered saline, pH 7.2, and i.n. inoculation into dams was made with 2.5×10^2 LD_{50} in 0.05 ml between Days 6 and 15 of gestation. LD_{50} was determined for suckling ICR mice inoculated intracerebrally (i.c.). For detecting infectious virus in tissue samples from dams, 2-day-old suckling ICR mice from an MHV-free breeder (Japan CLEA, Tokyo) were used.

Complement fixation(CF) test. The TG strain of SDAV, which was grown on LBC cells (8), was treated with diethylether and used in the CF test (6).

Histopathology: Tissues of the salivary gland, pregnant uterus, brain and other major organs were sampled from infected dams and foetuses as well as suckling mice, and they were fixed in phosphate-buffered formalin, pH 7.2. Paraffin sections were made and stained with hematoxylin and eosin (HE).For detecting SDAV antigen on tissue sections the avidin-biotin-complex(ABC) method (9) was applied using rabbit or mouse antiserum to MHV-2 sharing common antigens with SDAV (6,10,11) and a commercial kit (Stravingen, Bio-Genex, California). Anti-MHV rabbit serum was kindly supplied by Prof. N. Goto, Department of Veterinary Pathology, University of Tokyo. The results of the immunohistochemistry were confirmed by immunofluorescence (12) using fluorescein-conjugated goat antiserum to rabbit or mouse IgG (Serotec, Oxford).

RESULTS

After i.n. inoculation of SDAV into twenty 13-week-old female SHR rats, all the animals showed apparent clinical signs of sialoadenitis on Days 4 to 7 post-inoculation (p.i.)(Fig. 1). Through the course of observation one of them had a regular oestrous cycle of 4 days, but 19 of 20 (95%) were found to have signifcant disorder in the cycle starting on Days 0 to 10 p.i. and persisting for 6 to 18 days.

The next experiment was carried out to study effect of SDAV infection on pregnancy. Three groups of 7 female rats of age 14 weeks received i.n. inoculation of the virus 2, 3 or 4 days before gestation, while two groups of 7 rats received i.n. inoculation on Day 0 or 2 of gestation. Another group of 10 rats remained uninfected. On Day 14 of pregnancy all animals were sacrificed and examined for foetuses. As presented in Table 1 and Fig.2, 16 of 83 (19%), 33 of 68 (49%) and 10 of 44 (23%) foetuses were found aborted or dead in dams infected on Days -2, 0 and 2 of gestation, respectively. Only 7 of 108 (6%) foetuses were dead in non-infected dams. Similar results were obtained in another experiment using 19-week-old females (Table 1). In dams infected on Day 0 of gestation, 43 of 94 (46%) foetuses were aborted or dead on Day 14 p.i.

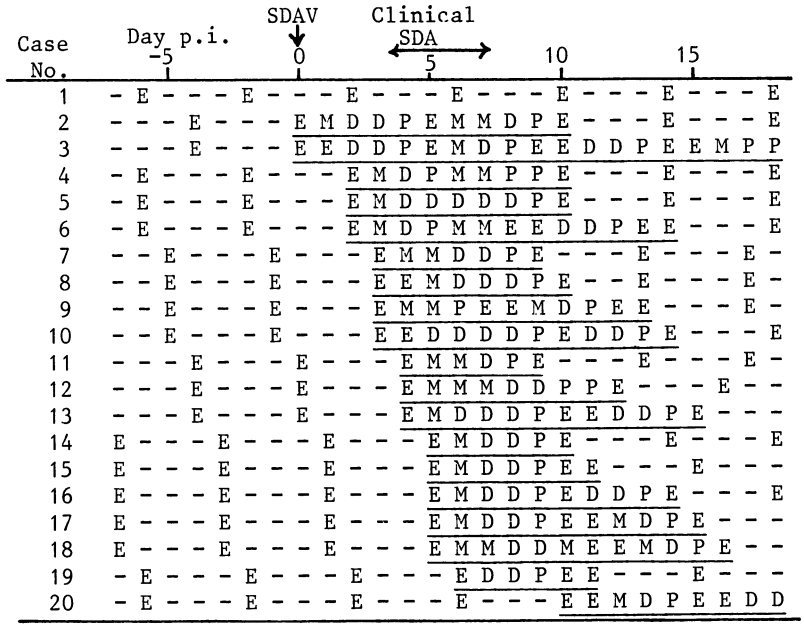

Fig. 1. Irregular estrous cycles in 13-week-old SHR rats infected with SDAV. P:Proestrus, E:Estrus, M:Metestrus, D:Diestrus, determined by vaginal smear examination.

Table 1. Mortality of embryos in SDAV-infected SHR dams

Age of dams	SDAV[a] (i.n.)	Number of dams		Number of implanted embryos[c]		
		Mated[b]	Gestated	Mean±S.D.	Total	Dead(%)
14W	Day -4 of gestation	7	7	7.7±4.1	54	8(15%)
	-3	7	7	10.9±1.4	76	4(5%)
	-2	7	7	11.9±1.5	83	16(19%)
	0	7	6	11.3±3.5	68	33(49%)
	2	7	5	8.8±5.2	44	10(23%)
	Not inoculated	10	10	10.8±1.6	108	7(6%)
19W	Day -5 of gestation	7	7	12.3±2.2	86	9(10%)
	-4	6	5	11.2±2.1	56	12(21%)
	-3	6	6	12.8±1.9	77	11(14%)
	-2	5	5	12.2±4.3	61	6(10%)
	0	8	8	11.8±2.0	94	43(46%)
	2	6	6	12.3±2.0	74	5(7%)
	Not inoculated	10	10	11.4±2.2	114	2(2%)

a) 2.5×10^2 LD_{50} for suckling mice(i.c.).
b) Positive for sperms on vaginal smear.
c) On Day 14 of gestation.

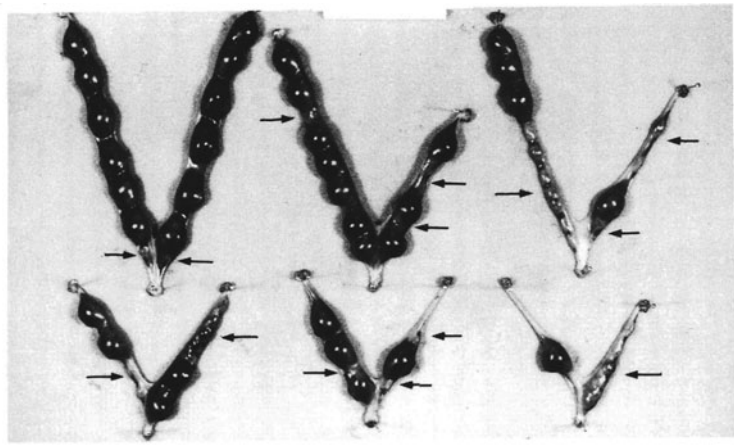

Fig. 2. Pregnant uteruses from dams inoculated i.n. on Day 0 of gestation, showing implanted, but dead or aborted embryos (arrows) present at Day 14.

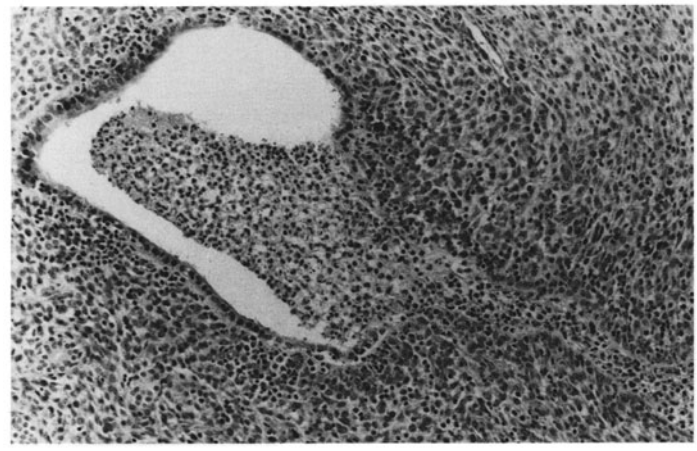

Fig.3. Exudative and desquamative endometritis in a dam infected on Day 0 of gestation.Day 6 p.i. HE stain.

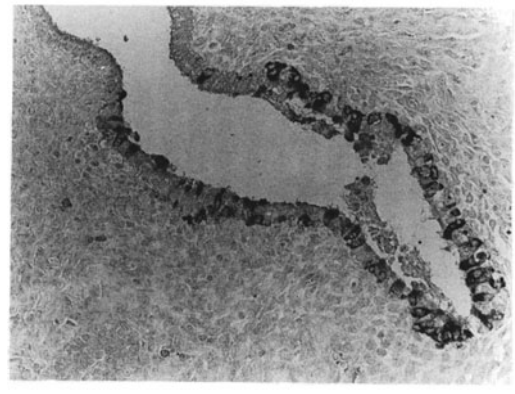

Fig.4. SDAV antigen within the endometrium of a dam infected on Day 0 of gestation. Day 6 p.i. Anti-MHV rabbit serum-ABC and methyl green stain.

Table 2. Effect of diet restriction on the mortality of embryos

SDAV[a] (i.n.)	Diet	Number of dams	Number of implanted embryos[b] Mean±S.D.	Total	Dead(%)
-	Restricted[c]	6	11.5±2.1	69	0
-	ad libitum	6	9.8±2.6	59	1(2%)
+	ad libitum	7	12.1±2.9	85	22(26%)

a) 2.5×10^2 LD_{50} for suckling mice(i.c.).
b) On Day 14 of gestation.
c) 4 g pellets per day for 4 days(Days 3 to 6 of gestation).

Table 3. Mortality of youngs from SDAV-infected SHR dams

Age of dams	SDAV[a] (i.n.)	Gestation period in days	Number of dams		Number of youngs		
			Delivered	Nursing	Litter size[b]	Total	Dead(%)[c]
13W	Day 2 of gestation	21,22	6	5	11.5	69	17(25%)
	5	22	6	5	11.7	70	14(20%)
	10	21,22	6	5	11.2	67	36(54%)
	15	23,24	4	0	8.8	35	35(100%)
	Not inoculated	22	5	4	11.0	55	19(35%)
16W	Day 2 of gestation	21-23	5	5	6.8	34	3(9%)
	5	22,23	6	5	8.7	52	5(10%)
	10	22,23	5	5	12.0	60	3(5%)
	15	22-25	5	2	9.0	27	13(48%)
	Not inoculated	22,23	6	5	11.2	67	15(22%)

a) 2.5×10^2 LD_{50} for suckling mice(i.c.).
b) Mean.
c) At 2 weeks of age.

To see whether the death of embryos described above resulted from SDAV infection *in utero*, 10 dams were inoculated i.n. with the virus on Day 0 of gestation, and they were sacrificed 5 or 6 days later and examined for pathology of the pregnant uterus. The uteruses from 3 dams were shown to have severe endometritis with desquamated endometrium and accumulation of neutrophils and mononuclear cells in the lumen (Fig.3). By immunohistochemistry SDAV antigen was detectable within the endometrium as well as exudate cells (Fig.4). By i.c. inoculation of affected uterine tissue into suckling mice, infectious virus was recovered which produced fatal encephalitis with viral antigen within neurons. Such viral metritis could not be produced in non-pregnant females, which had received the same virus inoculation and showed apparent sialoadenitis. Since food consumption was noticed to decrease during clinical mani-

festation of sialoadenitis, the effect of dietary restriction on the viability of embryos was studied. Nineteen females aged 13 weeks were mated with males, and 6 of them were given 4g of pelleted diet per day for 4 days between Days 3 and 6 of gestation. The remaining 13 rats were fed *ad libitum* and 7 of them received i.n. inoculation of SDAV on Day 0 of gestation. A11 the animals were sacrificed and examined for the number of dead embryos on Day 14 of gestation. As shown in Table 2, 22 of 85 (26%) embryos were found dead in SDAV-infected dams given full diet. However, there was no fetal death in dams with dietary restriction.

In the foregoing experiments, dams infected on Day 2 of gestation showed a lower rate of fetal death than those infected on Day 0 (Table 1). The next experiment was done to see the survival rate of sucklings from dams infected on Day 2 or later of gestation. Thirty females were divided into 5 groups of 6 animals after mating, and 4 groups were exposed to i.n. inoculation of SDAV while the remaing one remained uninoculated.

Clinical signs of sialoadenitis were apparent in all dams 4 to 9 days after virus inoculation, that is, on Days 6 to 11, Days 9 to 14, Days 14 to 19 or Days 19 to 24 of gestation in each infected group. As shown in Table 3, dams infected on Day 15 of gestation had a considerably decreased number of newborns and none of the pups survived for 2 weeks. In other groups which were infected at an earlier stage of gestation, the mortality of newborns was rather lower than in non-infected controls which showed unexpectedly high mortality in this experiment. No specific lesions were observed in any organs of sucklings from infected and non-infected dams. Dams in all the groups were shown to have CF antibody to SDAV at a titer of 1:160 or 1:320.

Similar results were obtained in another experiment using 16 week-old females, and 13 of 27(48%) sucklings from dams infected on Day 15 of gestation were found dead before 2 weeks of age.

DISCUSSION

The SHR rat is known to be difficult to breed and rear because of high susceptibility to infectious agents (13, 14). Natural SDAV infection in laboratory rats has been revealed to cause disorders in the oestrous cycle and to lower the gestation rate to 50% resulting in poor reproductive performance (4,11). In this study with SHR rats the disorder was shown to appear already at the first estrus after inoculation of SDAV, persisting for more than 6 days. The oestrous cycle was disturbed in 40% of Wistar and Sprague-Dawley rats after infection with SDAV (unpublished observation). In SHR rats, however, almost all infected females showed remarkably irregular cycles, persisting as long as 19 days.

When the virus was inoculated on the Day 1 of gestation, almost a half of embryos were found dead, possibly as a result of viremia and infectious metritis occurring at the early stage of embryonic development. Most embryonic deaths seemed to occur on Day 6 or 7 of gestation when the virus titer in the maternal blood circulation was reported to be declining (15). At this stage of infection, affected dams were shown still to have severe inflammation in the submaxillary glands with the presence

of a great deal of virus, which might be the source of viremia. Moreover, the feto-maternal communication in the uterus should be close enough to cause the involvement of embryos in the local inflammation. In the uteruses of infected dams, which had dead or aborted embryos, viral antigen was detectable within *in situ* and desquamated endometrial cells as well as in exudate cells in the lumen. Infectious virus was successfully recovered from the affected uterine tissue by inoculation into the brains of suckling mice. These findings suggested that the death or abortion of embryos in SDAV-infected dams might be due to viral metritis involving the embryos implanted shortly before the onset of maternal illness.

No correlation was found between embryonic death and decreased diet consumption of the dams having severe signs of sialoadenitis.

The embryonic death rate was lower when dams were infected before or shortly after the start of gestation than when infected at the start of gestation. The number of dead embryos was not so great in dams inoculated at a later stage in gestation. However the delivery of young was retarded and most newborns died within 2 weeks of birth. Such retarded delivery and delayed death of young might result from inadequate nursing by seriously diseased dams infected shortly before parturition. Some dams who delivered normally were shown to have high serum antibody titres and to protect their young by transfer of maternal antibody via milk as described previously (15,16).

Reproductive performance has been reported to be adversely affected in mice infected with MHV (1) as well as in the fowl infected with avian infectious bronchitis virus (17). In the case of SDAV infection in rats, early abortion due to viral endometritis is a feature of the virus pathogenicity which manifests itself as sialodacryoadenitis in adult animals.

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