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Respiratory Infection in Mice with Sialodacryoadenitis Virus, a Coronavirus of Rats

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Sialodacryoadenitis virus (SDAV), a coronavirus of rats, evoked both serum neutralization and complement fixation antibody responses when inoculated intranasally in mice. Weanling gnotobiotic CD-1 mice inoculated intranasally with $10^{3.0}$ mean tissue culture infective doses of SDAV remained asymptomatic. Virus was recovered from the nasopharynx, trachea, and lung from day 2 to day 7. Viral antigen was readily detected by indirect immunofluorescence in the lung but rarely in the nasopharynx. Infected mice developed interstitial pneumonia. Susceptible mice contact exposed to experimentally infected mice developed antibody to SDAV. Epizootiological studies indicated that retired breeder mice can have complement-fixing antibody to SDAV and mouse hepatitis virus (MHV) in the absence of MHV infection. These studies show that SDAV is infectious for mice and can be a pathogen for the respiratory system. Thus, SDAV infection of mice may be responsible for spurious seroconversions to MHV.

Sialodacryoadenitis is a common, highly infectious, naturally occurring disease of rats that is characterized by self-limiting necrosis and inflammation of mixed or serous salivary glands, lacrimal glands, and upper respiratory mucous membranes (2). It is caused by a coronavirus, sialodacryoadenitis virus (SDAV), which is antigenically related to mouse hepatitis virus (MHV) (1a). A second coronavirus of rats, Parker's rat coronavirus (PRCV) (3), also causes rhinitis and mild sialoadenitis and antigenically cross-reacts with SDAV and MHV (1, 1a, 3).

Recently, during routine serological monitoring of rodent colonies at Yale, complement-fixing (CF) antibodies to MHV and SDAV were detected in sera of retired breeder ICR mice. The colony was asymptomatic, mice were free from lesions, and neither MHV nor SDAV was recovered from their tissues. Additional studies were performed to determine whether SDAV is infectious for mice and whether SDAV infection produces spurious seroconversions to MHV.

MATERIALS AND METHODS

Mice. Specific-pathogen-free CD-1, NIH Swiss, or NGP-N mice, 4 to 8 weeks old, were obtained from the breeding colony, Division of Animal Care, Yale University School of Medicine. They were housed in plastic boxes with autoclaved pine chip bedding and filter lids and were fed food and water ad libitum. Axenic CD-1 male mice (6 to 8 weeks old; Charles River Breeding Laboratories, Wilmington, Mass.) were kept in a sterile, flexible-plastic isolator and were fed sterile food and water. Isolators were monitored repeatedly for bacterial and fungal contamination and remained sterile throughout the experiments.

Virus. Several SDAV strains were used for initial infectivity studies. The sources of these strains are given in Table 2. Strain 721176, previously used for pathogenetic studies in rats (1a), was used for the initial virus growth curve experiment in ICR mice and later for pathogenetic studies in axenic mice. This pool of SDAV was prepared from salivary glands of experimentally inoculated axenic rats as previously described (2). Virus titers were determined by inoculation of primary rat kidney cultures (1). The titer of the virus pool used for animal inoculation was $10^{5.0}$ mean tissue culture infective doses ($TCID_{50}$).

For convenience, details of preliminary infectivity studies are given in Results.

Antibody determinations. Serum-neutralizing (SN) and CF antibodies to SDAV and CF antibody to MHV were assayed as described previously (1a). Nonimmune serum and immune control sera of known titer were included in all tests.

Contact transmission. Thirty specific-pathogen-free mice were ear punched and inoculated intranasally (i.n.) with $10^{4.0}$ $TCID_{50}$ of SDAV. Twenty-four hours later they were distributed randomly, six per box, among five boxes, each containing two to five uninfected mice. Filter lids were used on all boxes, and care was taken to avoid cross-contamination. Three to four weeks postexposure, all mice were exsanguinated, and sera were tested individually for antibodies to SDAV.

Pathogenesis experiment. Forty axenic mice were inoculated i.n. with $10^{3.3}$ $TCID_{50}$ of SDAV, and virus quantitation of the inoculum was performed immediately. Mice were observed daily for clinical signs, and four mice were randomly selected for necropsy each day for 10 days. They were anesthetized

with sodium pentobarbitol and exsanguinated. Serum was stored at −20°C for antibody determination. The nasopharynx was irrigated with minimum essential medium in Hanks base containing 50% fetal bovine serum, 100 U of penicillin, and 100 mU of streptomycin per ml. Pieces of trachea, lung, salivary gland (submaxillary and parotid), and lacrimal gland (harderian and exorbital) were collected aseptically and stored at −60°C. Tissues and nasal washes were assayed for SDAV as previously described (2).

Sections of nasal turbinate, trachea, submaxillary and parotid salivary glands, and exorbital and harderian lacrimal glands were snap frozen on aluminum boats immersed in dry ice-alcohol baths. Lungs were inflated with 7.5% gelatin, held at 4°C for 1 h to gel, and then frozen on chucks at −20°C. Tissues were sectioned at 6 μm, fixed in cold acetone for 16 h, and stained by indirect immunofluorescence techniques as previously described (2). Sections were examined with a Zeiss microscope fitted with an HB0200 Osram lamp, a BG-12 ultraviolet exciter filter, and no. 44 and 53 barrier filters.

Transverse sections of nasopharynx at three levels and sections of trachea, lung (inflated with fixative via the trachea), cervical lymph node, salivary glands, lacrimal glands, heart, thymus, liver, spleen, kidney, pancreas, adrenal glands, gonad, eye, and brain were fixed in 10% neutral-buffered Formalin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined by light microscopy.

RESULTS

Antibody to SDAV in naturally and experimentally infected mice. CF antibodies to SDAV and MHV were detected in retired ICR breeder mice (Table 1). The pattern of seroconversion seemed atypical for MHV, since only several mice in each group had anti-MHV antibody.

In a separate experiment, antisera to SDAV were prepared by a single intraperitoneal or i.n. inoculation of NIH Swiss or NGP-N adult mice with various SDAV strains. By 3 to 4 weeks after inoculation, all groups of intraperitoneally inoculated mice had developed anti-SDAV CF and SN antibodies. Furthermore, all groups of i.n.-inoculated mice had SN antibody and five of eight had CF antibody to SDAV (Table 2), indicating that active infection had occurred. This was confirmed in a preliminary infectivity trial. Adult ICR mice were inoculated i.n. with $10^{3.0}$ $TCID_{50}$ of SDAV strain 721176. Infectious virus was recovered from the lung by day 2, and titers gradually decreased to undetectable levels by day 8. Small quantities of virus were also recovered from the trachea (Fig. 1).

In a second infectivity experiment, 30 ICR mice were divided into three groups of 10 mice each. One group was given $10^{3.5}$ $TCID_{50}$ of SDAV i.n., the second group was given two SDAV inoculations with a 2-week interval, and one group remained uninoculated. All mice were exsanguinated 4 weeks after the primary inoculation, and antibody titers to SDAV were determined. Sera from mice inoculated i.n. with SDAV either once or twice did not contain CF antibody to SDAV or MHV, but SN antibody to SDAV was detected.

TABLE 1. *CF antibodies to MHV and SDAV in ICR mice in a barrier colony*

Serum no.	Mouse		Date of collection	CF antibody titer to:	
	Age (months)	Sex		MHV	SDAV
1	12	F	2/5/73	TC[a]	8
2	12	F	2/5/73	AC[b]	>32
3	14	F	3/7/73	80	>32
4	14	F	3/7/73	40	8
5	14	M	3/7/73	<10	<4
6	10	M	3/7/73	<10	<4
7	16	M	3/7/73	<10	4
8	12	F	8/1/73	ND[c]	>32
9	14	F	8/1/73	ND	<4
10	20	F	8/1/73	ND	AC
11	12	F	8/1/73	ND	16
12	14	F	8/1/73	ND	4
13	17	F	8/1/73	ND	<4
14	14	M	8/1/73	ND	8
15	12	M	8/1/73	ND	4
16	14	M	8/1/73	ND	4

[a] TC, Serum reacts with control antigen.
[b] AC, Anticomplementary serum.
[c] ND, Not done.

Contact transmission experiment. Contact transmission of SDAV, as determined by seroconversion, occurred in all boxes of mice, and the ratio of experimentally infected mice to contact-exposed mice per box had no apparent effect on the efficacy of transmission (Table 3).

Pathogenesis. The pathogenesis of SDAV infection was characterized in axenic mice. Mice remained asymptomatic for 10 days after i.n. inoculation. SDAV was recovered from the trachea and lung by day 2 but was not detected in the respiratory tract after day 7 (Table 4). Virus was not recovered from salivary or lacrimal glands.

Viral antigen was also restricted to the respiratory tract and was most readily detected in the lung. Multiple small groups of alveoli contained interstitial and lining cells with cytoplasmic fluorescence. There were also small amounts of fluorescing debris in some alveolar spaces. The nasal and tracheobronchial mucosa generally did not contain viral antigen. However, viral antigen was detected in a short segment of bronchial epithelium from one mouse on day 5 and in a segment of nasal epithelium from one mouse on day 5.

Macroscopic lesions were limited to multiple, discrete, red-brown foci distributed evenly over all lobes of the lung. Histologically, several foci of epithelial necrosis were found in the nasal mucosa of one mouse, but lesions were otherwise

TABLE 2. *SN and CF antibody in pooled sera of mice 3 to 4 week after intraperitoneal (i.p.) or i.n. inoculation of SDAV*

SDAV		Mouse[a]		Inoculation route	Antibody	
Strain	Source	Strain	Age (weeks)		SN	CF
721400	DA rat	NIH Swiss	8–12	i.p.	+	10
		NIH Swiss	4–6	i.n.	+	<10
721432	DA rat	NIH Swiss	8–12	i.p.	+	≥40
			4–6	i.n.	+	20
721472	DA rat	NIH Swiss	8–12	i.p.	+	10
			4–6	i.n.	+	<5
721750	CD rat	NIH Swiss	8–12	i.p.	+	20
			4–6	i.n.	+	40
751175	Wistar/Lewis rat	NGP-N	5–7	i.n.	+	20
751177	Wistar/Lewis rat	NGP-N	5–7	i.n.	+	20
751181	Wistar/Lewis rat	NGP-N	5–7	i.n.	+	<5
751184	Wistar/Lewis rat	NGP-N	5–7	i.n.	+	10

[a] Six mice per group.

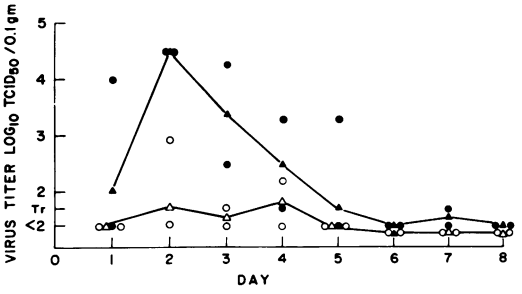

FIG. 1. *Virus titers in the lung (●, ▲) and trachea (○, △) of specific-pathogen-free outbred ICR mice after i.n. inoculation of 3.0 $\log_{10}$ $TCID_{50}$ of SDAV. (●, ○) Individual titer; (▲, △) average titer.*

TABLE 3. *Contact transmission of SDAV in mice*

Box	Inoculated[a]	Contact exposed
1	2/4	2/2
2	3/3	2/3
3	2/2	3/4
4	1/1	4/5
5	2/3	3/3

[a] Number seropositive/number in group.

restricted to the lung. The typical pulmonary lesion was multifocal interstitial pneumonia (Fig. 2 to 4). Inflammation began by day 3 and usually affected respiratory bronchioles and adjacent alveoli first. Alveolar septa were thick from infiltration by mononuclear cells. Alveolar lining cells, macrophages, lymphoid cells, neutrophils, and some necrotic cells were found in alveolar spaces. Foci spread to confluence as the infection progressed, and more-advanced lesions were accompanied by hyperplasia and hypertrophy of alveolar lining cells, with focal atelectasis. Pneumonic foci were still present by day 10, but they were less prominent, indicating that lesions were regressing.

DISCUSSION

These studies demonstrate, by several criteria, that SDAV is infectious and pathogenic for adult mice: (i) SDAV replicated in the respiratory tract; (ii) SDAV caused interstitial pneumonia; (iii) viral antigen was detected by immunofluorescence in affected lungs at about the same time that virus was recoverable; and (iv) mice developed antibody to SDAV after i.n. inoculation of small quantities of virus or after contact with infected cagemates.

SDAV was recovered from both upper and lower respiratory tracts of experimentally infected mice, but lesions were detected primarily in the lower respiratory tract. Other tissues were unaffected. Thus, the tissue tropism of SDAV in mice was different than in experimentally infected rats, in which necrosis and inflammation of submaxillary, parotid, and harderian glands and upper respiratory mucosa is common and interstitial pneumonia does not occur (2).

Virus was not recovered from all inoculated axenic mice in the pathogenesis study, nor were the lesions seen in all mice. It is possible that all mice were not infected because they were not anesthetized for inoculation in the isolator. In addition, all infected mice may not have developed lesions or lesions may have been transient. Since SN antibodies were detected in sera of all mice by postinfection day 8, virus replication may have been below the detection level in some animals.

All SPF mice developed anti-SDAV SN antibody within 3 weeks after experimental infection, whereas only 62% developed CF antibodies. In rats, the SN antibody response after experimental SDAV infection appears earlier and is of higher magnitude than the CF antibody re-

TABLE 4. *SDAV isolations from the respiratory tract of mice after i.n. inoculation with $10^{3.3}$ $TCID_{50}$ of SDAV*

Specimen	No. positive for virus/no. tested									
	1[a]	2	3	4	5	6	7	8	9	10
Nasal wash	0/3	0/3	1/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3
Trachea (pooled)	−	+	+	+	+	+	−	−	−	−
Lung	0/3	1/3	1/3	2/3	2/3	1/3	1/2	0/3	0/3	0/3

[a] Days post-inoculation.

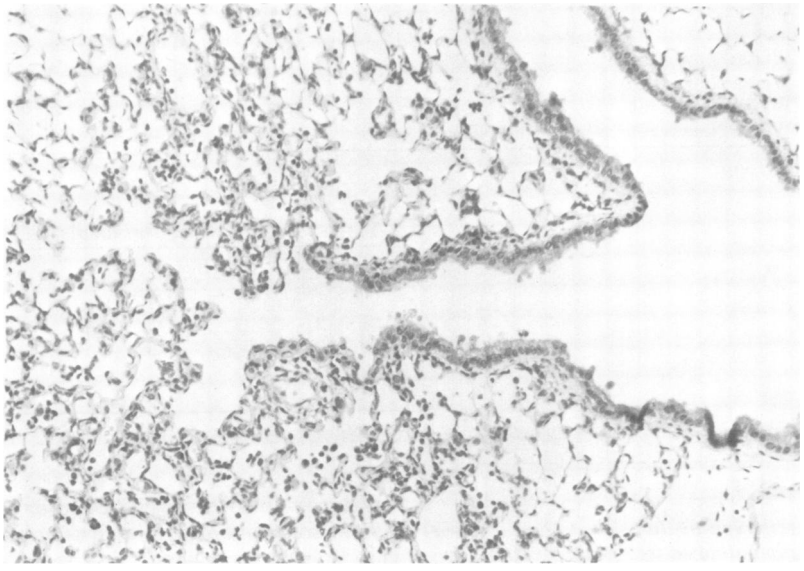

FIG. 2. *Interstitial pneumonia 4 days after i.n. inoculation of SDAV. Early pneumonic changes involve alveoli adjacent to terminal arborizations of bronchi.*

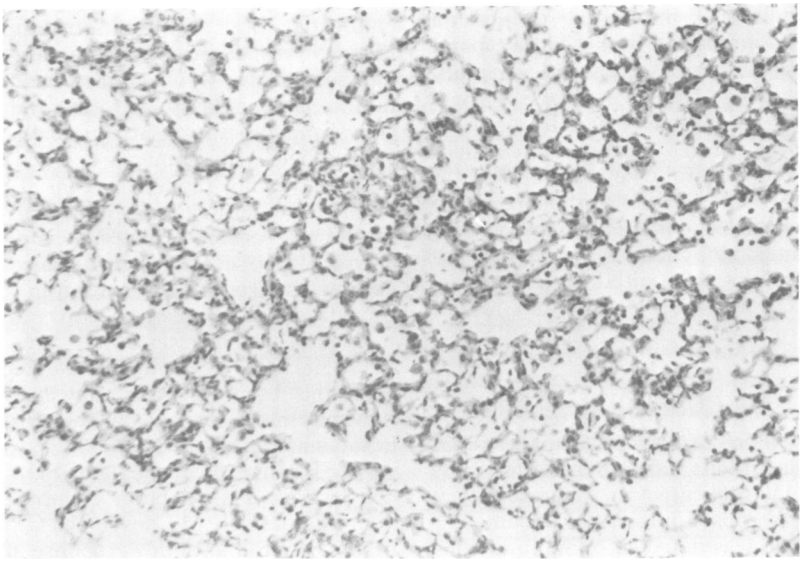

FIG. 3. *Low-magnification photograph of a typical area of interstitial pneumonia 4 days after i.n. inoculation of SDAV.*

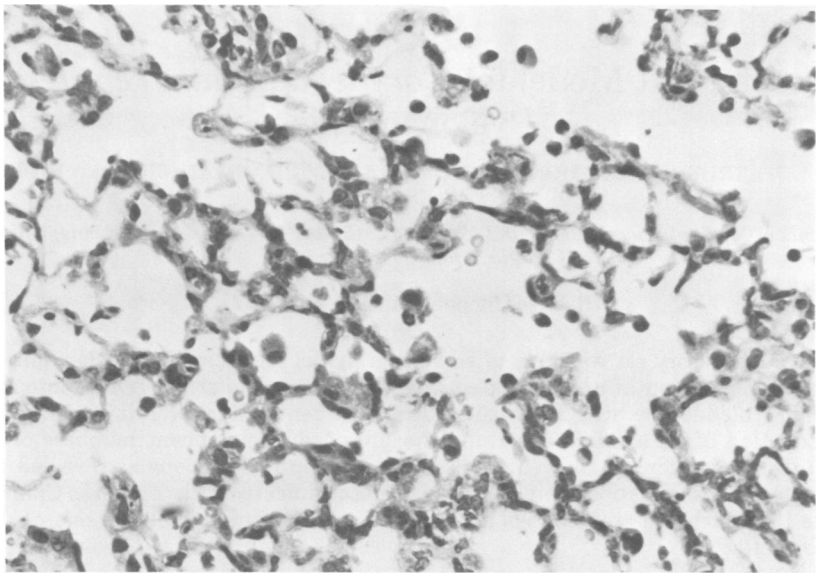

FIG. 4. *High-magnification photograph of a typical area of interstitial pneumonia 4 days after i.n. inoculation of SDAV. Alveolar septums are infiltrated by mononuclear cells. Some macrophages, neutrophils, and erythrocytes occupy alveolar spaces.*

sponse (P. N. Bhatt and A. M. Jonas, manuscript in preparation). The same may be true in mice, and CF antibody titers were probably below the level of detection. On the other hand, antibody responses may depend on the virus strain or mouse strain, since CF antibody to SDAV was detected in NIH Swiss and NGP-N mice that had been infected with different strains of SDAV. The age of mice may also be important: mice from the colony with CF antibody to SDAV and MHV were much older than those inoculated experimentally with SDAV.

In the multiple-infection experiment, the second inoculation was made after 2 weeks. It is likely that local immune response prevented reinfection and, thus, antigenic booster. This might explain why a CF antibody response was not detected. The particular virus or mouse strain used might have played a role also.

Detection of CF antibody to MHV in a mouse colony has generally been accepted as indicating MHV infection. MHV is a serious problem not only because infected animals are useless for experiments involving virological, immunological, or tumor work, but also because MHV can cause high morbidity and mortality. In addition, MHV carriers may spread infection to other colonies. In the colonies described in this report, it is likely that seroconversion to MHV and SDAV was caused by SDAV, because SDAV was present in the facility and mice were free from MHV by virological and morphological criteria. However, that an unusual MHV strain or an unidentified coronavirus may have caused this seroconversion cannot be ruled out without further studies.

These studies indicate the need for an improved serological test to distinguish between antibodies to MHV and SDAV. One can use the SN test, but it is too cumbersome to be used routinely. Furthermore, they reemphasize that it is important to isolate the viral agent elliciting an antibody response in a rodent colony to confirm the specificity of the antibody. The findings also indicate that additional host range and epizootiological investigations of SDAV will be required to determine its true spectrum of infectivity and pathogenicity for laboratory animals.

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