Exp. Anim. 37(3), 251—255, 1988

Reactivities of 4 Murine Coronavirus Antigens with Immunized or Naturally Infected Rat Sera by Enzyme Linked Immunosorbent Assay

Kenichi MACHII, Hiroshi IWAI, Yuko OTSUKA, Katsumoto UEDA and *Norio HIRANO

*Department of Veterinary Public Health, Institute of Public Health, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan, and *Department of Veterinary Microbiology, Iwate University, 3-18-8 Ueda, Morioka City, Iwate 020.*

(Received 30 October 1987/Accepted 19 February 1988)

Four murine coronavirus antigens, sialodacryoadenitis virus (SDAV) strain TG, Parker's rat coronavirus (PCV) strain 8190, mouse hepatitis virus (MHV) strains S and NuU, were examined for their reactivities to hyperimmunized and naturally infected rat sera by ELISA. With the immunized sera, SDAV and PCV antigens reacted best with respective homologous sera. MHV antigens reacted with all antisera, anti-SDAV, anti-PCV, and anti-MHV-S at approximately the same level, and MHV-S showed a slightly higher reactivity than MHV-NuU. The reactivities of the sera from various colonies to these antigens were in the order—from high to low—of SDAV, MHV-S, MHV-NuU, and PCV. None of sera negative for SDAV antigen reacted positively to the other antigens. Within the sera positive for SDAV, the positivities were in the order of MHV-S, MHV-NuU, and PCV. These results suggested that, although homologous antigens are best to detect SDAV or PCV infection by ELISA, MHV antigen can be used if highly cross-reactive viral strain is selected.

Sialodacryoadenitis virus (SDAV) and Parker's rat corona virus (PCV) have been reported to be coronaviruses which naturally infect rats [2, 3, 11]. For the serological monitoring of infections with coronaviruses in rats, some strains of mouse hepatitis virus (MHV) have been used because of their antigenic cross-reactivities among murine coronaviruses [1, 6, 8, 9, 12]. For the appropriateness of the usage of MHV antigen for detection of rat coronavirus antibodies, Smith [12] described that bivalent MHV-S/JHM antigen used in immunofluorescence test on infected rat sera for detecting SDAV antibody showed higher sensitivity than SDAV antigens used in complement fixation or neutralization test, and Iwai et al [6] showed correlation between incidence of CF antibodies to MHV and occurrence of SDA-like disease in rat colonies. As far as we know, there is no report concerning direct comparison of reactivity among SDAV, PCV, and MHV antigens with rat sera. In the present study, we compared the reactivities of 4 murine coronavirus antigens including SDAV, PCV, and 2 strains of MHV with immunized and naturally infected rat sera by enzyme linked immunosorbent assay (ELISA).

Materials and Methods

Viruses and preparation of antigens: SDAV strain TG and PCV strain 8190 were propagated in LBC cells [4, 5]. MHV strain S (MHV-S) and strain NuU (MHV-NuU) were provided by courtesy of Dr. Nakanaga,

Institute of Medical Science, University of Tokyo and propagated in DBT cells [3]. Antigens used in ELISA were prepared as follows. LBC cells were inoculated with SDAV and PCV, 10^5 50% tissue culture infective doses ($TCID_{50}$)/25 μl and 10^6 $TCID_{50}$/25 μl, respectively. DBT cells were inoculated with MHV-S and MHV-NuU 10^4 $TCID_{50}$/25 μl and 10^5 $TCID_{50}$/25 μl of viruses, respectively. The infected cells were collected by rubber policeman with culture fluids and were centrifuged for 10 min by 3,000 rpm at 5°C. The pellet was resuspended in phosphate-buffered saline (PBS) with 0.1% NaN_3 (NaN_3-PBS) and sonicated at 9 kHz and 200 W for 10 min, and after a centrifugation for 10 min by 3,000 rpm at 5°C the supernatant was stored at 4°C.

Antisera: All the rats used for preparing antisera to coronaviruses were female retired breeders of Sprague Dawley strain (Crj: CD) purchased from Charles River Japan, Inc. Anti-SDAV serum was prepared as follows. Five rats were infected with 50 μl of 10^5 $TCID_{50}$ SDAV intranasally (i.n.) followed by three i.n. booster inoculations of the same dose as primary immunization, at 14, 21, and 31 days and bled 6 days after the last inoculation. Five sera were pooled and stored at −20°C. For preparation of anti-PCV serum, two female rats were infected i.n. with 50 μl of 10^6 $TCID_{50}$ PCV followed by three i.n. booster inoculations of the same dose, at 7, 21, and 33 days and bled 4 days after the last inoculation. Sera were pooled and stored at −20°C. For anti-MHV-S serum, five female rats were inoculated i.n. with MHV-S, followed by intraperitonial booster inoculation at 14 days and bled after 7 days. These sera were pooled and stored at −40°C. Horseradish peroxidase conjugated anti-rat IgG rabbit IgG(HRPO-aRIgG) for ELISA was purchased from Miles Yeda Ltd. (Israel).

Test sera: Two hundred and six rat sera tested in the first survey were kindly supplied by Dr. Yamauchi, Kagoshima University, Dr. Maejima, Keio University, Dr. Koshimizu, University of Tokyo, Dr. Urano, Kumamoto University, Dr. Kawamoto, Tokyo Medical College (First survey). They were diluted into 1 : 10 with NaN_3-PBS for ELISA. In the second survey, additional 33 sera were obtained from 5 rat breeder colonies and they were diluted into 1 : 20 with NaN_3-PBS.

ELISA: Antigen plates were prepared as follows: 96-well polyvinylchloride microplates (Falcon, #3912, Bekton Dickinson, Co., Calif.) were coated with 30μl/well of optimally diluted antigen, and air dried. The procedure of ELISA using HRPO-aRIgG as the secondary antibody and 5 aminosalicylic acid as an indicator was carried out as described previously [7, 10].

Statistical analysis: The difference of positivity was examined by Chi-square test and the comparison of means were examined by Student's t-test.

Results

Reactivities of four antigens, SDAV, PCV, MHV-S,and MHV-NuU with hyperimmunized antisera to SDAV, PCV, and MHV-S are shown in Table 1. SDAV and PCV antigens reacted best with the homologous antisera, at the titers 1 : 2560 and 1 : 640, respectively. The MHV-S antigen reacted with homologous antiserum at 1 : 320 and with the other antisera at 1 : 640. MHV-NuU antigen reacted with anti-SDAV, anti-PCV, and anti-MHV-S at titers of 1 : 320, 1 : 640, and 1 : 160, respectively. Next, the reactivities of 4 coronaviral antigens with naturally occurring antibodies in rat sera were examined. In the first survey, 206 rat sera obtained from university facilities were tested (Table 2). Although positivities differed among the sources, 124 (60%), 73 (35%), 116 (56%), and 90 (44%) sera in total showed positive reaction with SDAV, PCV, MHV-S, and MHV-NuU antigens, respectively. Posi-

Table 1. Cross-reactivities of 4 antigens with 3 hyperimmune sera

Anti-serum to	Antigens			
	SDAV	PCV	MHV-S	MHV-NuU
SDAV	2560*	160	640	320
PCV	1280	640	640	640
MHV-S	80	20	320	160

*: Reciprocal of serum dilution

Table 2. Incidence of antibodies in rat sera* (first survey)

Group	Antigens			
	SDAV	PCV	MHV-S	MHV-NuU
A	10/10 (100)#	10/10 (100)	10/10 (100)	10/10 (100)
B	19/26 (73)	19/26 (73)	19/26 (73)	19/26 (73)
C	94/139 (68)	44/139 (32)	87/139 (63)	61/139 (44)
D	1/20 (5)	0/20	0/20	0/20
E	0/11	0/11	0/11	0/11
Total	124/206 (60)	73/206 (35)[1]	116/206 (56)	90/206 (44)[1]

*: Dilution at 1 : 10

#: Positive/Tested (%)

1: Difference was significant from SDAV, $p<0.001$.

Table 3. Reactivities of SDAV-positive sera to other antigens (first survey)

Antigen	No. of Serum	Antibody Titers								% of Positive
		10	20	40	80	160	320	640	1280<	
SDAV	124	23	15	22	12	17	19	10	6	100
PCV**	73	24	11	13	16	8	1	—	—	59
MHV-S*	116	40	11	12	11	18	8	11	5	93
MHV-NuU**	90	27	5	12	18	18	7	2	1	73

Mean antibody titers to SDAV, PCV, MHV-S and MHV-NuU antigens were 75, 20, 49, and 32 respectively.

Difference of positivity was significant from SDAV; **: $p<0.001$, *: $p<0.05$.

tivity between SDAV and MHV-S antigen was not significantly different ($p>0.05$), but the differences between SDAV and PCV or MHV-NuU were significant ($p<0.001$). All the sera positive for PCV, MHV-S, or MHV-NuU antigens were also positive for SDAV antigen. Namely, none of the sera negative for SDAV antigen reacted positively to the other 3 antigens. Table 3 shows reactivities of SDAV-positive sera with the other 3 antigens. Positivities for PCV, MHV-S, and MHV-NuU antigens were 59, 93, and 73%, respectively. The difference of positivity was significantly lower in PCV and MHV-NuU antigens ($p<0.001$), and in MHV-S antigen ($p<0.05$) than in SDAV. Among these SDAV-positive sera, the mean titer for MHV-S antigen (1 : 49) was significantly lower than that for SDAV antigen (1 : 75; $p<0.05$), and the mean titer of sera for PCV antigen (1 : 20) was significantly lower than those for SDAV or for MHV-S antigen ($p<0.001$).

Table 4. Incidence of antibodies in rat sera* (second survey)

Group	Antigens		
	SDAV	MHV-S	MHV-NuU
F	8/8 (100)#	7/8 (88)	8/8 (100)
G	8/10 (80)	6/10 (60)	4/10 (40)
H	5/5 (100)	4/5 (80)	1/5 (20)
I	1/5 (20)	1/5 (20)	0/5
J	5/5 (100)	2/5 (40)	0/5
Total	24/33 (82)	20/33 (61)	13/33 (39)[1]

*: Dilution at 1 : 10

#: Positive/Tested (%)

1: Difference was significant from SDAV, $p<0.01$.

In the second survey, additional 33 sera from rat breeder colonies were examined with SDAV, MHV-S, and MHV-NuU antigens

Table 5. Reactivities of SDAV-positive sera to other antigens (second survey)

Antigens	No. of Serum	Antibody Titers						% of Positive
		20	40	80	160	320	640	
SDAV	27	3	3	3	7	4	7	100
MHV-S*	20	9	3	3	4	1	—	74
MHV-NuU*	13	4	2	6	—	—	1	48

Mean antibody titers to SDAV, MHV-S, and MHV-NuU antigens were 160, 37, and 32 respectively.

*: Difference of positivity was significant from SDAV, $p<0.001$.

(Tables 4, 5). The positivity was a little lower than the first survey, 82% for SDAV antigen, 61% for MHV-S antigen, and 39% for MHV-NuU antigen. There were the same tendencies as in the first survey—the difference in positivity between SDAV to MHV-S antigen was not significant ($p<0.05$), but SDAV to MHV-NuU was significant ($p<0.01$). Similar to the first survey, none of sera negative for SDAV antigen reacted positively to the other 2 antigens, and 74 and 48% of SDAV-positive sera reacted with MHV-S and MHV-NuU, respectively. The positivity was significantly lower in MHV-S antigen ($p<0.05$) and in MHV-NuU antigen ($p<0.001$) than in SDAV antigen. Mean antibody titer for MHV-S antigen (1 : 37) was significantly lower ($p<0.001$) than that for SDAV antigen (1 : 160).

Discussion

MHV is widely used for detection of antibodies to coronaviruses such as SDAV and PCV in rat sera because of its cross-reactive nature. The present data showed that MHV-S antigen reacted with immune rat sera against SDAV and PCV as well as with the homologous antiserum, in contrast to strain-specific reactivity of SDAV and PCV antigens, indicating a relatively high potency of MHV-S antigen to detect antibodies to coronaviruses of the rat (Table 1). In the survey of naturally occurring antibody, positivity with MHV-S was not significantly different from that with SDAV; in contrast, with the other antigens, significantly lower positivity than with SDAV antigen was seen (Tables 2, 4). Nevertheless, positivity with MHV-S in the SDAV-positive sera was significantly lower than that with SDAV. At the same time, antibody titers with MHV-S were lower than those with SDAV (Tables 3, 5). However, it seems that, in spite of the difference of antibody-detection rate between SDAV and MHV-S antigens, the potency of MHV-S antigen to detect up to 93% of SDAV-positive sera is sufficient for routine use in monitoring SDAV infections in rat colonies.

The results from the present survey which showed that there was no PCV-positive sera in SDAV-negative sera and that higher reactivities of sera were seen to SDAV than to PCV (Tables 2, 3), indicate that the coronavirus infections in rats are mainly caused by SDAV and that PCV infection may be rare among rat colonies.

In conclusion, to detect serum antibodies to SDAV or PCV, the use of SDAV and PCV antigens is recommended, but if the strain is carefully selected, MHV may be sufficient for use in seromonitoring.

This work was supported by Grants-in-Aid for Scientific Research from the Agency of Science and Technology and from Ministry of Education, Science and Culture (No. 60780069) of Japan.

References

[1] Bhatt, P. N., Percy, D. H., and Jonas, A. M. (1972). Characterization of the virus of sialodacryoadenitis of rats: A member of the coronavirus group. *J. Infect. Dis.*, **126**, 123-130.

[2] Eisenbrandt, D. L., Hubbard, G. B., and Schmidt, R.E. (1982). A subclinical epizootic of sialodacryoadenitis in rats. *Lab. Anim. Sci.*, **32**, 655-659.

[3] Hirano, N., Fujiwara, K., Hino, S., and Matsumoto, M. (1973). Replication and plaque formation of mouse hepatitis virus (MHV-2) in mouse cell line DBT culture. *Arch. Ges. Virusforsch.*, **44**, 298-302.

[4] Hirano, N., Ono, K., Inoue, A., Murakami, T. and Takamaru, H. (1985). Replication of rat coronavirus in a rat cell line, LBC. *Arch. Virol.*, **75**, 301-304.

[5] Hirano, N., Takamaru, H., Ono, K., Murakami, T., and Fujiwara, K. 1986. Replication of sialodacryoadenitis virus of rat in LBC cell culture. *Arch. Virol.*, **88**, 121-125.

[6] Iwai, H., Itoh, T., Kagiyama, N., and Nomura, T. (1980). Monitoring of murine infections in facilities for animal experimentation. pp. 219-222. *In* Animal Quality and Models in Biomedical Research, Spiegel, A., Erichsen, S., and Solleveld, H. A.

(edit.), Gustav Fischer Verlag, Stuttgart.
[7] Iwai, H., Yamaguchi, R., Otsuka, Y., Ueda, K., and Saito, M. (1984). Immunoglobulin classes of anti-Sendai virus antibody detected by ELISA in infected nude mouse serum. *Microbiol. Immunol.*, **28**, 489-491.
[8] Kraft, V. and Meyer, B. (1986). Diagnosis of murine infections in relation to test methods employed. *Lab. Anim. Sci.*, **36**, 271-276.
[9] Lussier, G., and Descôteaux, J.-P.(1986). Prevalence of natural virus infections in laboratory mice and rats used in Canada. *Lab. Anim. Sci.*, **36**, 145-148.
[10] Machii, K., Otsuka, Y., Iwai, H., Ueda, K., Suzuki, E., Saito. M., and Nakagawa, M. (1985). Evaluation of enzyme-linked immunosorbent assay (ELISA) in diagnosis of Mycoplasma pulmonis infection in rats. *Jpn. J. Vet. Sci.*, **47**, 845-848.
[11] Parker, J. C., Cross, S. S., and Rowe, W. P. (1970). Rat coronavirus (PCV): A prevarent, naturally occurring pnuemotropic virus of rats. *Arch. Ges. Virusforsch.*, **31**, 293-302.
[12] Smith, A. L. (1983). An immunofluorescence test for detection of serum antibody to rodent coronaviruses. *Lab. Anim. Sci.*, **33**, 157-160.
[13] Utsumi, K., Ishikawa, T., Maeda, T., Shimizu, S., Tatsumi, H., and Fujiwara, K. (1980). Infectious sialodacryoadenitis and rat breeding. *Lab. Anim.*, **14**, 303-307.

4種類のネズミ由来コロナウイルス抗原に対するラット血清の反応について

町井研士・岩井　浤・大塚佑子・上田雄幹・*平野紀夫

国立公衆衛生院衛生獣医学部
*岩手大学農学部家畜微生物学教室

ELISA によるラット血清中の抗コロナウイルス抗体検出のために，唾液腺涙腺炎ウイルス（SDAV）TG 株，パーカーのラットコロナウイルス（PCV）8190株，及びマウス肝炎ウイルス（MHV）S 及び NuU 株で作製した抗原の，免疫血清及び自然感染血清との反応性を比較検討した。免疫血清についての検討では，SDAV 及び PCV 抗原は，同種抗原に対する抗血清と最も高い反応性を示した。一方，MHV 抗原はすべての抗血清と同程度の反応性を示し，また，MHV-S の方が MHV-NuU より高い反応性を示した。数カ所のラット飼育集団由来の自然感染血清と各抗原との反応性は，SDAV，MHV-S，MHV-NuU，PCV という順に高い傾向を示し，SDAV 陰性の血清で他の抗原に陽性のものは認められなかった。また，SDAV 陽性の血清は，MHV-S，MHV-NuU，PCV の順に陽性率が低下する傾向がみられた。これらの結果より，ELISA によるラットのコロナウイルス抗体の検出には SDAV および PCV 抗原の使用が最適であるが，ウイルス株によっては MHV 抗原も利用し得ることが示唆された。