Some Antigenic Relationships of the Murine Parvoviruses: Minute Virus of Mice, Rat Virus, and H-1 Virus[1] (36088)

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Minute virus of mice (MVM) (1), rat virus (KRV) (2), and H-1 virus (3) are members of the parvovirus group, and each occurs as a natural infection in laboratory rodents: MVM in mice and perhaps rats (4, 5), KRV in rats (6, 7), and H-1 in rats (6, 8, unpublished data). Fragmentary evidence from the literature (1, 5, 9, 10) suggests that the rodent parvoviruses may be serologically distinct, while other studies indicate some degree of antigenic sharing (1). Thus the serologic relatedness of these viruses is unclear. Studies on the antigenic relationships of the three rodent parvoviruses by fluorescent-antibody (FA), tissue culture neutralization, complement-fixation (CF), and hemagglutination-inhibition (HI) tests are reported below, and the diagnostic value of each test for detection and identification of virus and virus antibody is evaluated.

Materials and Methods. MVM virus was obtained from Dr. L. V. Crawford, Imperial Cancer Research Fund, London, England. Kilham strain of rat virus (KRV) was provided by Dr. J. Hartley, National Institutes of Health, Bethesda, MD; and H-1 virus (Toolan) was purchased from the American Type Culture Collection. Virus pools were prepared in either primary or secondary rat embryo cell cultures (RECC). For FA and neutralization tests RECC were planted at cell concentrations of approximately 1×10^5 cells/ml and inoculated 24 hr later when the cultures were still subconfluent. For the production of HA and CF antigens, confluent RECC cultures were inoculated. Each virus pool was examined for extraneous viruses by the rat and/or mouse antibody production test (11).

Fischer germfree rats used for the preparation of cell cultures and antisera were purchased from Charles River Breeding Laboratories, Wilmington, MA. This breeder colony has been tested for several years without evidence of infection with MVM, KRV, or H-1 virus. Rats used to produce antibody were housed within germfree isolators. For preparation of hyperimmune sera, rats were inoculated three times by the intraperitoneal route at 1 week intervals with 1 ml of cell-free fluid from infected RECC and were bled the end of week 4. Similarly, a control antiserum was prepared with uninfected RECC. A pool of serum from uninoculated rats also was used as a control. The reactivity of the control reagents, if any, was slight, and did not interfere with the reading or interpretation of results presented herein.

For the FA test, glass cover slips with monolayers of RECC were infected with 10^6 $TCID_{50}$ of either MVM, KRV, or H-1 virus. Cover slips were harvested 72 hr after inoculation and stained (11). The rabbit antirat globulin was purchased from Microbiological Associates, Inc., and by HI and FA tests shown to be free of antibody to KRV, H-1 virus, and MVM. Lissamine rhodamine counterstain was supplied through the courtesy of Dr. Roger Wilsnack, Huntingdon Research Center, Baltimore, MD.

The microtiter technique (12) was used for hemagglutination (HA), HI, and CF tests (4, 13). One percent guinea pig red blood cells suspended in saline containing 0.25% bovine plasma albumin were used in the HA and HI tests; and tests were run at room temperature. Eight HA units were used in HI tests. The CF tests employed 3 CH_{50} units of complement and overnight incubation at

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4°. Complement-fixing antigens were prepared by infecting confluent monolayers of RECC with the appropriate virus, and cultures were harvested when approximately 50% of the cells showed cytopathic effects (CPE). Uninoculated cultures handled in a similar manner were used as controls. The cells were suspended in 1/20 vol of the culture fluids, mixed with an equal volume of undiluted receptor-destroying enzyme, incubated overnight at 37° and heated at 56° for 30 min. The CF antibody titers were determined from block titrations so that the antibody titers reflected the reaction which occurred at the optimal concentration of antigen. Control antigen did not react with any of the antiserum pools.

For the neutralization test, 1000 tissue culture infectious doses in 0.1 ml were diluted with an equal amount of serum, incubated at 37° for 1.5 hr; and the 0.2 ml was inoculated into a roller tube of RECC. Tubes were rocked for 1 hr and fed with 0.8 ml of medium. Cell cultures were examined for CPE from days 5 through 9.

Results. The results of this study are presented in Table I. MVM, KRV, and H-1 virus did not cross by HI or CF at a 1:10 serum dilution (lowest dilution tested). Although both the CF and HI tests can be used to distinguish the three rodent parvoviruses, the HI tests are faster, less expensive, and easier to perform. Also, the CF antigen must be concentrated in order to obtain a quantity of antigen optimal for detection of antibody.

No cross reactivity occurred in the neutralization tests; but, compared to the homologous control, the CPE caused by H-1 virus in RECC were delayed by 1 to 2 days by MVM antisera of both mouse and rat origin. The cell state of the tissue cultures influenced the outcome of neutralization tests (11). The neutralization tests could be run for KRV and H-1 viruses with confluent RECC cultures; however, with subconfluent cultures CPE were observed earlier and the tests were completed sooner with an equal number of infectious doses. For the MVM neutralization test with 1000 $TCID_{50}$ doses, CPE were observed in 3 to 5 days in the antigen control, so the test was completed in a week. However, in confluent cultures CPE were not apparent for 14 to 21 days; and, by this time, the RECC cultures were not acceptable for evaluating neutralization.

By FA the three rodent parvoviruses were found to share a common antigen(s). MVM virus was stained by both H-1 and KRV antibody, but MVM antibody did not stain KRV or H-1. Both nuclear and cytoplasmic fluorescence were observed for each homologous reaction; and the fluorescence seen in the cross reactions was not distinguishable from the FA stain observed in the homologous reactions. As observed previously for MVM (11), nuclear and cytoplasmic fluorescence in cultures infected with KRV or H-1 did not occur in the same cell. The FA was more sensitive for antibody detection than the CF or HI tests, but since the rodent parvoviruses shared common antigen(s) KRV, H-1, and MVM viruses cannot be differentiated by the FA test.

Discussion. The rodent parvoviruses, MVM, KRV, and H-1, do not cross react in CF, HI, or neutralization tests, but they do

TABLE I. Serologic Relationship Between Minute Virus of Mice (MVM), Rat Virus (KRV), and H-1 Virus.

	Antibody titer by serologic test											
	FA			HI			CF			Neutralization		
Antigen	MVM	KRV	H-1	MVM	KRV	H-1	MVM	KRV	H-1	MVM	KRV	H-1
MVM	1280[a]	160	160	320	—[b]	—	160	—	—	160	—	—
KRV	—	1280	160	—	320	—	—	320	—	—	≥2560	—
H-1	—	320	1280	—	—	320	—	—	640	—	—	2560

[a] Reciprocal of antibody titer.
[b] — = <10.

share a common antigen(s) which is demonstrable in FA tests. The FA test appears to be a very sensitive test for detection of virus and virus antibody, while the HI, neutralization, and CF tests are suitable for establishing the definite identity of the rodent parvoviruses.

Crawford (1), in his description of MVM as a new parvovirus, noted a kaolin removable, one-way cross where rat virus hemagglutinin was slightly inhibited by MVM antibody. In the present study, no low-level cross was observed in either HI or CF tests. Other relationships of the parvoviruses have been noted in work reported by other investigators. Moore (9) and Nicholson and Hetrick (10) have shown that KRV and H-1 virus are antigenically distinct by HI tests. Kilham and Margolis (5) have shown that MVM resembles KRV and H-1 in being more pathogenic for hamsters than for mice or rats and producing a lethal disease when inoculated into neonatal hamsters; however, KRV and H-1 antisera do not afford protection from disease when suckling mice are inoculated with hamster-passaged MVM. Minute virus of canines (MVC) antiserum does not react with H-1 virus, KRV, or MVM, and homologous titers of the rodent parvoviruses were at least 32-fold higher than titers of MVC although some low-level crosses were observed (15). Hoggan (14) has shown that AAV types 1, 2, 3, and 4 are serologically distinct by CF, tissue culture neutralization, and FA tests except for low-level crosses between types 2 and 3.

KRV, H-1, and MVM are found frequently in laboratory rodents (4–8). Although the natural history is not known accurately, the following points may be made. KRV was originally isolated from rat tumors (2) and isolations from naturally infected rats followed (7). There have been no indications that this virus may have another host besides the rat. MVM was a contaminant in a pool of mouse adenovirus (1); the prevalence of virus antibody in mouse colonies and the isolation of virus from normal mice has been documented (4, 12, 16). Antibody to MVM has been found to be prevalent in rat sera, but the evidence that the antibody resulted from MVM infection is not conclusive; however, the data are considered as evidence that rats are natural hosts for MVM or a related agent (5, 12). H-1 virus isolations have been reported from human tumor materials (3) and human embryos (17); and HI and neutralizing antibody had occasionally been detected in human sera (18, 19). The credibility of the data, however, has been questioned (18–20), and in more recent studies investigators were unable to detect antibody or isolate virus from similar materials (20). Also, we have been unable to detect H-1 HI antibody in the sera of 50 laboratory technicians who have frequent contact with the virus (unpublished data). Thus the relationship of the virus to human infection is not clear. Antibody to H-1 virus is found frequently in wild and colony reared rats (6, unpublished data) and Kilham and Margolis (8) have isolated the virus from uninoculated rat organ cell cultures. Also, we have isolated the virus from kidneys, livers, and lungs of normal rats (unpublished data). No parvovirus has been isolated from hamsters, and there is no evidence from antibody studies that these rodents are natural hosts for KRV, H-1, or MVM (unpublished data). Many guinea pig sera, however, have a nonspecific hemagglutination inhibitor which is removed by treatment with receptor-destroying enzyme (unpublished data). It is concluded from available information that MVM is prevalent in mice and perhaps rats, and KRV and H-1 virus are prevalent in rats. These rodents and cell cultures from their tissues are important to research in virology. The presence of these viruses may be a serious problem, particularly since it is evident that they may be introduced into an experiment or laboratory without previous design. It is important to understand their relationship, and what diagnostic tools are available for their study. The FA test is a sensitive diagnostic tool for rapid detection of KRV, H-1, and MVM, and the HI, neutralization, and CF tests are good tests for specific identification.

Summary. Minute virus of mice, rat virus, and H-1 virus share one or more antigens by immunofluorescence, but are antigenically-distinct by hemagglutination-inhibition, com-

plement-fixation, and tissue culture neutralization tests.

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