Gazdar Strain of Murine Sarcoma Virus. Biologic and Antigenic Interactions with the Heterologous Hamster Host[1] (36582)

PADMAN S. SARMA, ADI F. GAZDAR, HORACE C. TURNER, AND PICHITRA DEJ KUNCHORN

National Cancer Institute and Microbiological Associates, Bethesda, Maryland 20014

The presently known strains of murine sarcoma viruses (MSV) (1–3) induce sarcomas in certain heterologous hosts including hamsters and rats (4–12). MSV induced hamster sarcomas and tissue culture cell lines of MSV transformed hamster cells usually do not contain detectable quantities of infectious or noninfectious virus but contain defective MSV genome rescuable in techniques using helper leukemia viruses (6, 10, 13). However, the species-specific group-specific antigen (gs-1 antigen) of the viruses of the murine leukemia–sarcoma complex (6, 7, 14–17) which serves as an antigenic marker for the viruses of this group, is not present in detectable amounts in such hamster cells. Therefore, the sera of MSV tumor bearing hamsters do not contain detectable levels of antibodies against the gs-1 antigen of the mouse leukemia viruses (MuLV). However, in certain cases, MSV induced hamster tumors yield infectious virus (12, 18–20) but such viruses are tropic for hamsters and are the result for the interaction of the defective MSV genome with endogenous hamster C-type viruses resulting in the formation of MSV pseudotypes. Such pseudotype viruses have the viral envelopes and gs-1 antigen of the hamster leukemia virus (19, 20). The hamster tumors and tissue cultures infected with this hamster pseudotype virus contain the gs-1 antigenic marker of the hamster but not the mouse C-type viruses.

The MSV strains and accompanying helper mouse leukemia viruses are known to interact with the heterologous rat host to produce sarcomas, most of which contain a mouse tropic virus and/or gs-1 antigen of the mouse C-type viruses (1–4, 6, 8, 11). The presence of this antigen is attributable to the capacity of the MuLV helper virus to replicate to detectable levels in such rat tumors. Thus, rats bearing primary or transplanted sarcomas generally develop antibodies against gs-1 antigen of the mouse C-type viruses. Serum pools containing high titers of MuLV-specific antibodies serve to detect the noncytopathogenic mouse leukemia viruses in a tissue culture complement-fixing (CF) antigen induction test, the COMUL test (11), analogous to the COFAL test for avian leukosis viruses (21).

Recently, we described the isolation of a new strain of MSV (22–24). This strain, designated Gazdar MSV (abbreviated to Gz-MSV), was isolated from a naturally occurring sarcoma of a New Zealand White $\times$ New Zealand Black F_1 hybrid mouse. The virus induced sarcomas in mice as well as in hamsters, rats and *Mastomys*. Virus stocks of Gz-MSV contained an associated MuLV which appeared to function as a helper for virus replication. The mouse passage virus had the envelope antigenic characteristics of viruses of the FMR subgroup.

This report describes our recent observations which suggests that this new strain of MSV differs from the three presently known strains of MSV (1–3, 6, 7, 12, 18, 19) in its ability to induce both gs-1 antigen and the corresponding antibodies in the hamster host, despite its failure to mature into infectious virus in such hosts. The sera of tumor-bearing hamsters containing CF antibodies satisfactorily served for the CF detection of the murine leukemia gs viral antigens as well as infectious virus by the COMUL test. This induction of gs antigen and corresponding

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antibodies in hamsters appears to be largely due to the presence in the hamster tumors and cultures derived from such tumors of a noninfectious C-type virus with the properties of a sarcoma virus (25).

Methods and Results. Virus. Virus stocks of Gz-MSV were prepared as partially purified concentrates of mouse tumors (6, 26). Virus titers were established by focus assay in NIH Swiss strain mouse embryo fibroblast (MEF) cultures and tumor assay in newborn mice of this strain.

CF test. The group-specific antigens of the mouse and other C-type viruses and their corresponding antibodies were quantitated by a standard micro CF procedure, previously described (4, 11, 21).

Induction of sarcoma in hamsters. Newborn Golden Syrian hamsters were inoculated subcutaneously on the day of birth with 0.1 ml amounts of virus containing $\geq 10^6$ MEF focus-forming units. Sarcomas developed at the site of the inoculation 21 to 45 days after inoculation. Unless sacrificed, the majority of animals succumbed within 60 days from massive tumors with or without distant metastases (22). The tumors had the histological characteristics of undifferentiated sarcomas and were similar in appearance to sarcomas induced in hamsters by the Moloney strain of MSV (M-MSV) (10, 27). The tumors were readily transplanted into newborn and weanling hamsters. Such inoculated hamsters developed progressively growing sarcomas and they generally died within 6 weeks after transplantation. Transplanted tumors of a few animals underwent spontaneous regression; such animals were resistant to subsequent tumor transplantation.

Absence of infectious virus in hamster tumors. Partially purified concentrates (6, 26) of primary and transplanted hamster tumors were free of demonstrable infectious MSV or MuLV as tested by inoculation into newborn BALB/c and NIH Swiss mice and by COMUL tests *in vitro* in cultures of NIH Swiss MEF and Syrian hamster embryo fibroblasts (4, 11). Electron microscopic examination of primary and transplanted hamster tumors and tissue culture cell lines (HTG1 and HTG2) derived from transplanted tumors showed mature and budding C-type virus particles (22, 25).

Detection of murine leukemia gs antigen. Clarified 20% tumor homogenates of primary and transplanted hamster tumors as well as

TABLE I. Murine Leukemia-Sarcoma Group-Specific Antigen in Hamster Tumors, Cultured Hamster Tumor Cells and "Noninfectious" Virus Derived from Tumor Cells.

Antigen specimen	CF antigen titer vs antisera[a]			
	M-MSV rat serum	MuLV gs-1 guinea pig serum	Gz-MSV hamster serum	HaLV gs-1 guinea pig serum
Tumor 20% extract	16–32[b]	>8	16–32	<2
HTG1 cell line 20% cell pack	>32	>4	>32	<2
HTG2 cell line 20% cell pack	>32	>4	>32	<2
Virus concentrate from HTG2 cell line	16	4	16	<2

[a] Antisera used: M-MSV rat serum = pool of sera of rats bearing transplanted Moloney MSV rat tumors; MuLV gs-1 serum = guinea pig antiserum against the gs-1 antigen of Rauscher leukemia virus prepared by the isoelectric focusing technique. HaLV gs-1 serum = guinea pig antiserum against purified gs-1 antigen of the hamster leukemia virus, prepared similarly. The guinea pig antisera were kindly provided by Dr. R. V. Gilden. Gz-MSV hamster serum = serum of a hamster bearing transplanted Gz-MSV hamster tumor.

[b] Reciprocal of highest dilutions giving 3 to 4+ complement fixation.

TABLE II. Group-Specific Reactivity in Complement-Fixation Test of Sera of Gz-MSV Tumor-Bearing Hamsters with Homologous Hamster and Heterologous Murine Sarcoma Virus Rat Tumor Antigens.

	CF antibody titer vs antigen.[a]					
Hamster serum no.	M-MSV[b] rat pool 5	Gz-MSV mouse 29435	Gz-MSV hamster 29949	Gz-MSV hamster 29950	Polyoma hamster T-1523	Polyoma mouse S-131A2
S3808	>80[c]	>80	>80	>80	<20	<20
S3809	>80	>80	>80	>80	<20	<20
S3810	40	80	80	>80	<20	<20
S3811	>80	>80	>80	>80	<20	<20
S3812	>80	>80	>80	>80	<20	<20
S3813	40	40	40	40	<20	<20
S3724	320	>80	>80	>80	<20	<20
S3725	>80	>80	>80	>80	<20	<20

[a] Tumor antigens were prepared as clarified 20% extracts.
[b] M-MSV rat = Moloney MSV rat antigen.
[c] Reciprocal of highest dilution giving 3 to 4+ complement fixation.

20% cell packs of the HTG1 and HTG2 cell lines of monolayer cultures contained high titers of the MuLV species-specific (gs-1) antigens (14–17) (Table I). The antigen was also detected in ether disrupted, concentrated and purified virus, derived from supernatant fluids of HTG2 culture by double density gradient centrifugation. Possible contamination of such hamster preparations with an endogenous hamster C-type virus was excluded by testing these hamster tumor and viral preparations for CF reactivity against antisera containing the species-specific gs antibodies of the hamster C-type viruses (20) (Table I).

Development of CF antibodies against viral gs antigen. Hamsters which survived for over 21 days with primary or transplanted tumors generally developed demonstrable CF serum antibodies against the gs antigens of the mouse C-type viruses present in M-MSV rat tumors and purified and disrupted Rauscher leukemia virus preparations (Tables II and III). The same sera also reacted with hamster and mouse preparations of the homologous Gz-MSV but not against preparations of hamster tumors induced by unrelated viruses such as SV_{40} and polyoma viruses (Table II). The sera also failed to react with other MSV hamster tumor preparations which were free of demonstrable gs antigens of the murine leukemia viruses.

The progressive development of the described serum CF antibodies in hamsters bearing transplanted Gz-MSV tumors is shown in Table III. Antibody titers revealed by M-MSV rat tumor antigen and purified Rauscher leukemia viral antigens were approximately equal.

A high titered hamster antiserum (titer, 1:320) gave an equivalent positive CF reaction against 8 CF antigen units of the murine leukemia gs-1 antigen, prepared by the isoelectric focusing technique (16).

Hamster CF antibodies for COMUL test. The Gz-MSV hamster antisera gave positive CF reactions with tissue culture cell pack antigens of cultures infected with various strains of murine leukemia viruses. A high titered hamster serum (titer, 1:320) diluted to contain 8 CF antibody units efficiently served to detect and assay the Rauscher, Friend, Moloney and Gross leukemia viruses by the CF antigen induction test in NIH Swiss mouse embryo fibroblast cultures (COMUL test) (11).

Discussion. The Gz-MSV differs in one important respect from the presently known strains of MSV, the Moloney MSV (1, 6, 7, 13), Harvey MSV (2, 9, 13) and Kirsten MSV (3, 8, 13) in that the Gz-MSV virions obtained from hamster tumors, appear to

TABLE III. Progressive Development of Viral Group-Specific Complement-Fixing Serum Antibodies in Hamsters with Progressively Growing Transplanted Gz-MSV Hamster Tumors.

Hamster no.	Antigen[a]	CF antibody titers weeks after transplantation 3	4	5	7
1	Tumor	80[b]	80	80	D[c]
	Viral	80	>80	>80	
2	Tumor	40	40	>80	>80
	Viral	40	40	>80	80
3	Tumor	40	20	D	
	Viral	40	40		
4	Tumor	80	40	80	>80
	Viral	80	20	80	80
5	Tumor	80	>80	80	D
	Viral	80	>80	>80	
6	Tumor	20	20	D	
	Viral	20	20		
7	Tumor	<20	20	D	
	Viral	<20	20		
8	Tumor	<20	20	D	
	Viral	<20	20		

[a] Tumor antigen used was M-MSV rat pool 9, 20% clarified tumor extract. This antigen contains a high titer of murine leukemia species-specific gs antigen. Viral antigen used was mouse tissue culture derived Rauscher leukemia virus concentrated and purified by density gradient techniques and disrupted with Tween ether. Eight to 16 units of tumor and viral antigens were used in the CF test.

[b] Reciprocal of highest dilution giving 3 to 4+ complement fixation.

[c] D = dead with massive tumor growth.

contain the species-specific gs-1 antigen of the viruses of the murine leukemia group. Thus, Gz-MSV hamster tumors and cultures derived from such tumors, contained the gs-1 antigen as well as rescuable Gz-MSV sarcoma viral genome and noninfectious C-type virus particles (24, 25). MuLV was not detectable in such hamster preparations. The noninfectious C-type particles derived from the HTG1 and HTG2 cell lines contained the gs-1 antigen and this hamster derived murine virus had the biologic characteristics of a sarcoma virus as demonstrated by cocentrifugation with MuLV (25). Recently, we observed that rescue of the gs-1 antigen containing Gz-MSV from hamster tumor cells with C-type viruses of heterologous hosts such as the feline leukemia virus and the hamster leukemia virus results in the production of pseudotype viruses with the species-specific antigenic determinants of both interacting viruses (28).

Studies hitherto performed on other strains of MSV have shown that a nonproductive interaction of the defective MSV with various host cells including the homologous mouse (29) and heterologous rat (30, 31) and hamster (6, 7, 12, 18, 19) cells is evidenced by the incorporation of rescuable MSV genome in the resulting transformed cells (6, 27–31). However, such cells contain no detectable gs-1 antigen of the mouse C-type viruses (Table IV). The MSV pseudotype viruses rescued with C-type viruses of cats (13) or hamsters (12, 18–20) thus do not contain this mouse gs-1 antigenic marker. At present, it is not clear as to whether these mouse sarcoma virus strains lack the genetic information to code for the gs-1 antigen of the mouse C-type viruses or whether the synthesis of this antigen is repressed under certain conditions, such as association with heterologous host and heterologous C-type viruses.

The mouse passage Gz-MSV has the envelope antigenic characteristics of MuLV of the FMR subgroup (24). This property may reflect the envelope characteristics of the noncytopathogenic MuLV found in association with the sarcoma virus. The "helper" virus has not been detected in Gz-MSV hamster tumors or in cell lines derived from these tumors. The envelope property of the Gz-MSV hamster sarcoma virus and the possible occurrence of envelope antibodies in Gz-MSV tumor-bearing hamsters is unknown and is currently under study.

A practical application of the studies reported herein is the development and use of hamster antisera against the gs-1 antigen of the murine leukemia viruses, which are useful in the CF detection of murine C-type viral antigens and infectious virus. High titered antisera were readily produced by induction of primary as well as transplanted tumors in the hamsters and these antisera

TABLE IV. A Comparison of the Biological and Antigenic Characteristics of Viruses Recovered from Hamster Tumors Induced by Various Strains of Murine Sarcoma Viruses.

Hamster tumor induced by	Production of C-type virus[a]	Presence of MSV genome	Infectivity of virus recovered		Presence of gs-1 antigens	
			Mouse cells	Hamster cells	Mouse	Hamster
Moloney-MSV	—	+	—	—	—	—
(M-MSV)	+	+	—	+	—	+
Harvey-MSV	—	+	—	—	—	—
(H-MSV)	+	+	—	+	—	+
Kirsten-MSV	—	+	—	—	—	—
(Ki-MSV)	+	+	—	+	—	+
Gazdar-MSV	+	+	—	—	+	—
(Gz-MSV)	+	+	—	+	+	+

[a] Virus production was determined by electron microscopy and by the incorporation of ^{3}H-uridine into virus particles released into the medium of cultured tumor cells. The virus producing tumors of M-MSV, Ki-MSV and H-MSV are all hamster leukemia pseudotypes of MSV and contain an associated hamster C-type virus. These viruses are infectious for hamster cells but not mouse cells. Most Gz-MSV hamster tumors and cultured hamster tumor cells studied contained noninfectious C-type virus particles; antigen preparations derived from these contained the mouse but not the hamster gs-1 antigens. However, a subclone of HTG2 tumor cell line derived after *in vivo* passage in hamsters was recently found to contain both mouse as well as hamster gs-1 antigens, suggesting that a hamster leukemia pseudotype of Gz-MSV was present. Thus far, a nonproducing state of the Gz-MSV genome in hamster cells has not been encountered in several primary and transplanted Gz-MSV hamster tumors studied.

functioned as efficiently as M-MSV rat sera (6, 11) for the routine detection and assay of noncytopathogenic mouse leukemia viruses in the COMUL test (11).

Summary. The biologic and antigenic interactions of a new strain of mouse sarcoma virus, Gazdar MSV (Gz-MSV), with the heterologous hamster host are described.

Hamster tumors as well as cell lines derived from hamster tumors contained noninfectious C-type virus particles. The species-specific gs-1 antigen of the murine leukemia viruses was detected in various hamster tumor and tissue culture preparations including preparations of purified virus. Hamsters bearing primary or transplanted Gz-MSV hamster tumors consistently developed high titers of CF antibodies against the homologous Gz-MSV tumor antigens as well as against purified preparations of mouse leukemia viruses. The gs antisera from such hamsters were found to be as useful as the presently used M-MSV rat sera for the detection of the murine leukemia gs viral antigens and infectious virus by the COMUL test.

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