Antigenic Comparison of Rauscher Murine Leukemia Virus Cultivated in Human Embryo and Mouse Cells (36065)

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(Introduced by J. J. Oleson)

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Until recently the infection of nonmurine cell lines with murine leukemia and sarcoma viruses had not been reported. The infection of a diploid human embryonic lung culture, WI-38, with the Moloney strain murine sarcoma virus has been described (1). The successful infection of several human embryonic cell lines with the Rauscher murine leukemia virus has been reported (2). The infection of human cell lines by oncogenic RNA viruses derived from animals poses the question of the relationship of viral etiology of animal cancers to the potential viral etiology of human cancers. Here we wish to describe the antigenic alterations found in the Rauscher virus produced from an infected human embryo cell line HEK-1, as compared to the virus produced by a mouse cell line.

Materials and Methods. Mixed agglutination testing. The mixed agglutination test (3) was used to verify the species of origin of the infected cell cultures. Guinea pig anti-human (O+) erythrocyte serum, absorbed with sheep and BALB/c mouse erythrocytes was prepared as the human cell sensitizing serum. Rat anti-BALB/c mouse erythrocyte serum, absorbed with sheep and human (O+) erythrocytes, was prepared as the mouse cell sensitizing serum. Fresh pooled BALB/c erythrocytes and 21–25 day old human (O+) erythrocytes were used as indicator cells. Uninfected HEK-1 cells and (parallel) infected and uninfected JLS-V9 mouse bone marrow cells (4) were employed as controls. The purity of the infected cells was also verified by karyotyping.

Antisera. Antisera to the human cell-derived Rauscher virus (HRV), and to virus recovered from the plasma of leukemic mice, were produced in rabbits by injection of 1.0 ml of an equal mixture of virus and complete Freund's adjuvant distributed to four foot pads. This was followed 28 and 35 days later by intramuscular injections of 0.5 ml of virus. The animals were bled 2 weeks following the final injection. Crude serum of both types was found, initially, to be reactive with host cell components. This reactivity was eliminated prior to testing. The rabbit anti-HRV serum was multiply absorbed with a frozen and thawed suspension of HEK-1 cells and fetal calf serum until no reactivity for either was detectable by the test methods (described below). The anti-plasma RV and group specific sera were similarly absorbed with BALB/c mouse liver powder and plasma to eliminate nonspecific activity. Group specific antisera to murine leukemia viruses were prepared in rats by subcutaneous implantation of Moloney sarcoma in Fisher rats (designated MSS) (5) or by subcutaneous implantation of Rauscher virus induced lymphoid tumor in Osborne-Mendel rats (designated RSS).

Preparation of viruses. The HEK-1 human embryonic kidney cell line, initiated in our laboratories, was infected with virus harvested from cell-free, filtered, tissue culture supernate of the Rauscher virus infected human embryonic muscle cells, as previously described (2).

The viruses produced by the chronically infected HEK-1 cells (HRV) and JLS-V9 cells (V9-RV) were harvested from the tissue culture fluid and concentrated by ultracentrifugation to provide approximately 1 ×

10^{10} particles/ml in citrate or Veronal buffer. A Rauscher virus concentrate was similarly prepared from pooled plasma of leukemic BALB/c mice by ultracentrifugation using the Moloney technique (6). The virus preparation for the virus agglutination test consisted of freshly harvested virus in 0.05 *M* sodium citrate. Those virus preparations used for complement fixation and immunodiffusion were treated with ether, by the method described by Nowinski *et al.* (7).

Preparation of cellular antigens. Antigens for complement-fixation testing were prepared in the following manner. Freshly harvested cells were washed three times and resuspended to 10% in Veronal buffer. The cell suspension was sonicated on ice for three 30 sec cycles in a Mullard ultrasonicator. The sonicate was clarified by low speed centrifugation (600*g*, 15 min), and stored at —110°.

For immunodiffusion testing, cellular antigens were prepared by the following method. Cells were washed three times, then resuspended to a 10% suspension in phosphate buffered saline (pH 7.3). The suspension was frozen and thawed six times, then homogenized in a Virtis homogenizer. The homogenate was sonicated for three 30 sec intervals in a Mullard ultrasonicator, centrifuged at 200*g* for 15 min, then at 50,000*g* for 1 hr. The remaining supernatant fluid was concentrated 10-fold by dialysis in Aquacide (Calbiochem) and stored at —110°.

Immunologic test methods. HRV, V9-RV, plasma-derived RV, and preparations from the infected cells were compared immunologically by virus agglutination (8), complement fixation (microtiter) (9), immunofluorescence (10), or microimmunodiffusion (11).

Results. The results of the mixed agglutination test show that both the infected and uninfected HEK-1 cells are human and the cultures contain no detectable murine cells. The JLS-V9 cells are murine and the cultures contain no detectable human cells. The karyotypic analysis shows the experimentally infected cells to be of human origin.

In the complement-fixation test, the virus preparations from JLS-V9 and HEK-1 cells and saline antigens from the infected and non-infected cells were compared using the group specific (MSS and RSS) sera. No significant differences were found (Table I) between the virus or the cellular preparations either when sera were tested against 4 units of antigen or when antigen was tested against 4 units of antibody. These findings are supported by the results of indirect immunoflourescence examinations of acetone-fixed cell smears (Table II). The fluorescence reactivity is specific for control and experi-

TABLE I. Results of Complement-Fixation Testing of Virus and Cellular Antigens Derived from Rauscher-Infected Human and Murine Cells.

	RSS[a]		MSS[b]	
	Antibody titer[c]	Antigen titer[d]	Antibody titer[c]	Antigen titer[d]
Virus from				
HEK-1	1024	1024	256	2048
HEK-1 (ether treated)	1024	1024	256	2048
JLS-V9	512	512	256	1024
JLS-V9 (ether treated)	512	512	256	1024
Cellular antigen				
HEK-1, infected	256	32	256	64
control	Neg	Neg	Neg	Neg
JLS-V9, infected	128	32	256	128
control	32	Neg	Neg	8

[a] Rauscher rat sarcoma serum.
[b] Moloney rat sarcoma serum.
[c] Titered against 4 units of antigen.
[d] Titered against 4 units of antibody.

TABLE II. Results of Indirect Immunofluorescence Examination of Human and Murine Cells Infected with Rauscher Virus.

	Reactivity[a] with:		Antirat globulin conjugated control
	RSS	MSS	
HEK-1			
infected	70	70	0
control	0	0	0
JLS-V9			
infected	80	70	0
control	0	0	0

[a] Percentage cells fluorescence positive.

mental Rauscher virus-infected cells. The noninfected HEK-1 and JLS-V9 cells are nonreactive. However, no significant difference in the level of reactivity for the different types of infected cells is observed by this test.

The immunodiffusion comparisons of the viruses are presented in Figs. 1–3. The specific rat serum (MSS) (Fig. 1) produces two common lines of precipitation with both viruses plus an additional line with the V9-RV. No reactivity is observed with cellular antigens prepared in parallel from either JLS-V9 or HEK-1 cells indicating that the reactivity is virus associated. Rabbit antiserum to the plasma Rauscher virus produces one common line of reactivity between the two viruses and another specific for the V9-RV (Fig. 2). The rabbit antiserum to the HRV also produces a common line of reactivity between the two viruses. In addition, a line of reactivity is observed specifically related to the homologous human cell-derived HRV and is not shared by the murine V9-RV (Fig. 3). Neither rabbit serum shows reactivity for antigen from uninfected murine or human cells. Comparison of the rabbit antisera to the group specific rat sera shows that the antibodies responsible for the common reactions between the two different viruses are of the same specificity. The unshared reactivities are observed only between each rabbit antiserum and its homologous virus.

The virus agglutination test was applied to both viruses in a further attempt to detect and confirm differences in the viral coats. The results (Table III) support the conclusions drawn from immunodiffusion analysis. The group specific rat sera do not agglutinate either virus, and thus do not contain antibodies reactive to either viral coat. In con-

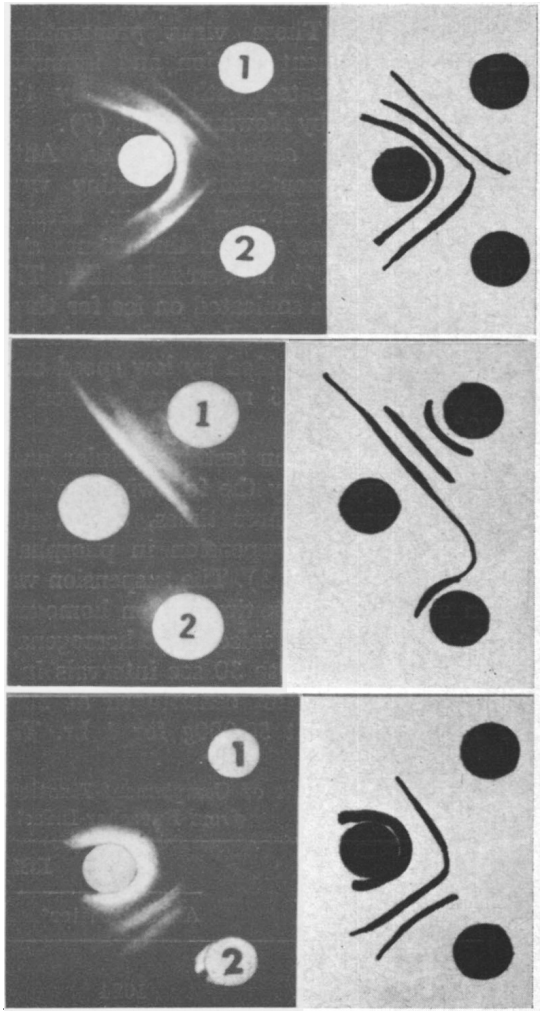

FIG. 1. Immunodiffusion comparison of V9-RV (1) and HRV (2) reactivity with the MSS murine leukemia group specific serum: JLS-V9 and HEK-1 extracts were nonreactive with either group specific serum.

FIG. 2. Immunodiffusion comparison of V9-RV (1) and HRV (2) reactivity with the antiplasma Rauscher virus serum: JLS-V9 and HEK-1 extracts were negative.

FIG. 3. Immunodiffusion comparisons of V9-RV (1) and HRV (2) reactivity with the anti-HRV serum: JLS-V9 and HEK-1 extracts were negative with this serum.

TABLE III. Results of Virus Agglutination Testing of Human Cell and Mouse Cell-Derived Rauscher Viruses.

Immune serum[a]	Virus	Result
Anti-mouse Rauscher virus	JLS-V9-RV	Pos
	HEK-1-RV	Neg
Anti-HEK-1 Rauscher virus	JLS-V9-RV	Neg
	HEK-1-RV	Pos
Moloney sarcoma rat serum	JLS-V9-RV	Neg
	HEK-1-RV	Neg

[a] Diluted 1:5.

trast, antiserum to the HRV agglutinates only the homologous virus and not the V9-RV. The antiserum to the mouse plasma virus, while nonreactive with the HRV, agglutinates the V9-RV and plasma RV. This indicates, in each, the presence of antibodies specific for the homologous virus coat.

Discussion. We have presented a serological comparison of human cell-derived Rauscher leukemia virus (HRV) to mouse cell-derived Rauscher virus (V9-RV). Contrary to the preliminary report (2), no significant differences between HRV and V9-RV were detectable by complement fixation or immunofluorescence using murine leukemia group specific rat sera. By immunodiffusion analysis it was evident, in reactions with the group specific rat sera, that the viruses share certain antigens. In view of the lack of activity of the group specific sera in the virus agglutination tests, it is believed that the shared antigen is associated with the core of the virus. Each virus also has specific nonshared antigens, demonstrated by immunodiffusion and by virus agglutination only with the homologous (rabbit) antisera. Neither virus was agglutinated by the heterologous antiserum. These nonshared antigens, detected only by reactivity with the homologous antisera, are believed to be components of the outer membranous coats of the viruses. The antigens appear to be related to antigenic modifications in the host cell membranes, in view of lack of cross-reactivity or of reactivity with uninfected host cell antigens. This may be analogous to the *in vivo* alteration of Kirsten murine sarcoma virus in hamsters reported by Klement *et al.* (12).

As was reported by Wright and Korol (2), the human cell derived Rauscher virus is not leukemogenic for BALB/c mice. However, no attempt was made to establish the immune status of mice so inoculated. In view of the antigenic similarities between the HRV and the leukemogenic V9-RV, studies have been initiated to determine if inoculation with the live, nonleukemogenic HRV will protect mice against subsequent challenge with the leukemogenic plasma-derived Rauscher virus. Preliminary results in a small group of animals have shown that some protection is afforded by early passaged virus as evidenced by a lower incidence of leukemia in the experimental group, following challenge, than in the control group.

Summary. We have compared antigens of Rauscher leukemia virus (RV) derived from a human cell line with antigens of RV grown in mouse cells. Group specific rat antisera (GSS) to murine leukemia viruses show no reactivity with the outer membranous coat of either type of virus. The GSS demonstrates that the core of the disrupted HRV retained the murine leukemia group specific antigens. Rabbit antisera, prepared against each virus, showed specificity for the homologous virus reflecting antigenic differences in the outer membranous coats.

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