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Assay For Type C Virus in Mouse Sera Based on Particulate Reverse Transcriptase Activity

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Assay of particulate reverse transcriptase activity in the sera from feral mice naturally infected with type C virus provides a sensitive and rapid procedure for the determination of in vivo virus infection. The results compare well with assays for infectious virus and with complement fixation or competitive radioimmunoassays for the p30 internal antigen of the virus.

The presence of the reverse transcriptase or RNA-dependent DNA polymerase (RDP) within the extracellular RNA tumor virions (1, 14) permitted the detection and approximate quantitation of these viruses in cell culture fluids of a variety of experiments related to virus induction, inhibition, stimulation, infection, and neutralization. We have utilized this virion-associated RDP activity in determining viremia in feral mouse populations by assaying this enzyme activity in particulate fractions of mouse sera. The results were compared with other existing methods such as complement fixation tests (10) of spleen extracts and competitive radioimmunoassays (3) of sera for the 30,000-molecular-weight (p30) internal antigen of the virus and assays for infectious virus in sera by induction of complement fixation p30 antigen in cell cultures (COMUL test) (10). It was shown earlier that about 85% of normal adult mice of a natural colony (LC) of wild mice (*Mus musculus*) have high titers of infectious type C viruses in their viscera and sera (5, 6), whereas the remaining 15% of adult LC mice have little or no detectable infectious virus. The assay described thus provides a rapid and useful means for identifying virus-positive and -"negative" LC wild mice for epidemiological screening purposes.

Portions (0.1 to 0.2 ml) of serum samples were diluted to 5 ml with STE buffer (0.1 M NaCl-0.01 M Tris-hydrochloride [pH 7.4]-0.001 M EDTA). After initial centrifugation at 10,000 × *g* for 10 min, the particulate materials were pelleted from the supernatant fluids by centrifugation at 84,000 × *g* for 45 min in a SW50.1 rotor. The pellet was suspended in 60 μl of a detergent solution containing 0.04% Nonidet P-40, 0.01 M Tris-hydrochloride (pH 7.4), 0.005 M dithiothreitol, and 0.03 M KCl. Portions (25 μl) of this suspension were assayed for RDP activity with a synthetic primer-template. The final concentration of reaction components (in 50 μl) were Tris-hydrochloride (pH 8.1), 0.05 M; KCl, 0.06 M; $MnCl_2$, 0.003 M; dithiothreitol, 0.0025 M; Nonidet P-40, 0.02%; [^{3}H]dTTP, 10 μCi (18 Ci/mmol); and 2 μg of $(dT)_{12-18}$·poly(rA). The reactions were incubated at 37°C for 1 h in small test tubes covered with Parafilm and then cooled in ice bath. Twenty-five-microliter portions of the reaction mixture were applied on a moist spot of 10 μl of 10 mM ATP on the center of a Whatman no. 1 filter paper disk (2.4 cm in diameter). After a few seconds when the liquids had spread on the filters, they were dropped into a solution of ice-cold 5% tricarboxylic acid containing 2% sodium pyrophosphate. After 20 min of equilibration, the filters were removed and placed into another ice-cold solution of 5% tricarboxylic acid and 1% sodium pyrophosphate for 10 min. After that the filters were further washed to reduce the background radioactivity by sequential removal and dipping in the following solutions: 5% tricarboxylic acid, 5% tricarboxylic acid, 5% tricarboxylic acid, 100% ethanol, and 100% ethanol. This succession of washings was carried out in ice-cold solutions with at least 10 ml of solution per filter and a duration of 5 min in each wash. Filters were then dried under a heat lamp and counted in 10 ml of scintillation fluid (8.0 g of butyl-PBD and 0.5 g of PBBO in 1 liter of toluene) in a Beckman LS 230 liquid scintillation counter.

To establish the validity of the assay, various amounts of wild-mouse type C virus, strain 292 (6), were added to NIH Swiss mouse sera shown free of particulate RDP activity. The data in Fig. 1 clearly indicate a direct relationship between the amount of added virus and the extent

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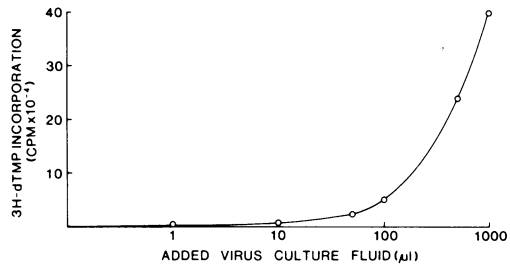

FIG. 1. *Detection of added virus in NIH Swiss mouse sera by assay of RDP. NIH mouse sera (0.2 ml) were mixed with designated amounts of culture fluids from wild-mouse 292 virus-producing NIH cell cultures. The mixture was diluted to 5 ml with STE buffer and centrifuged at 10,000 × g for 10 min, and then the virus was pelleted from the supernatant fluids. The enzyme activity in the virus pellets was determined as described in the text. Background values (100 to 300 cpm) obtained in the absence of the primer template were deducted from cpm values shown.*

of the enzyme reaction. Next several serum samples from NIH Swiss mice and from LC and BC mice (another wild-mouse colony with very low natural overt infection by type C viruses [5]) were assayed for particulate RDP activity. The data (Fig. 2) suggest that LC mice which showed detectable amounts of virus by COMUL and complement fixation also showed in general a significant RDP activity. Similarly, serum samples from NIH Swiss mice and from LC and BC wild mice which were negative by other tests also showed no or very low enzyme activity. The sera of 40 LC virus-positive mice gave a geometric mean of 13,888 cpm (range of 1,054 to 431,928 cpm), whereas LC virus negative (28 mice), BC (14 mice), and NIH Swiss (8 mice) showed geometric means of 96 cpm (range of 0 to 3,183 cpm), 45 cpm (range of 0 to 1,024 cpm), and 2 cpm (range of 0 to 527 cpm), respectively.

To confirm that the incorporation of label is specifically due to RDP activity and not due to the utilization of $(dT)_{12-18}$·poly(rA) by other cellular DNA polymerases, it was necessary to determine the primer-template specificity and to characterize the products of the endogenous reactions of the particulate serum RDP. Three serum samples from LC virus-positive mice were tested for particulate RDP activity with $(dT)_{12-18}$·poly(dA) and $(dT)_{12-18}$·poly(rA) as primer templates in the presence of Mg^{2+} as the divalent metal ion using the reaction conditions described by Robert et al. (12). The low ratio of $(dT)_{12-18}$·poly(dA) activity to $(dT)_{12-18}$·poly(rA) activity obtained with the serum samples was similar to that using purified LC wild-mouse type C virions and also consistent with previously published data on viral RDP (2, 8, 12; Table 1). Bacterial and mammalian cell DNA polymerases with Mg^{2+} as the divalent cation usually prefer $(dT)_{12-18}$·poly(dA) over $(dT)_{12-18}$·poly(rA) as primer template (2, 8, 12). Additional evidence regarding the relationship of the serum RDP to mouse type C virus RDP was obtained by determining the effect of anti-

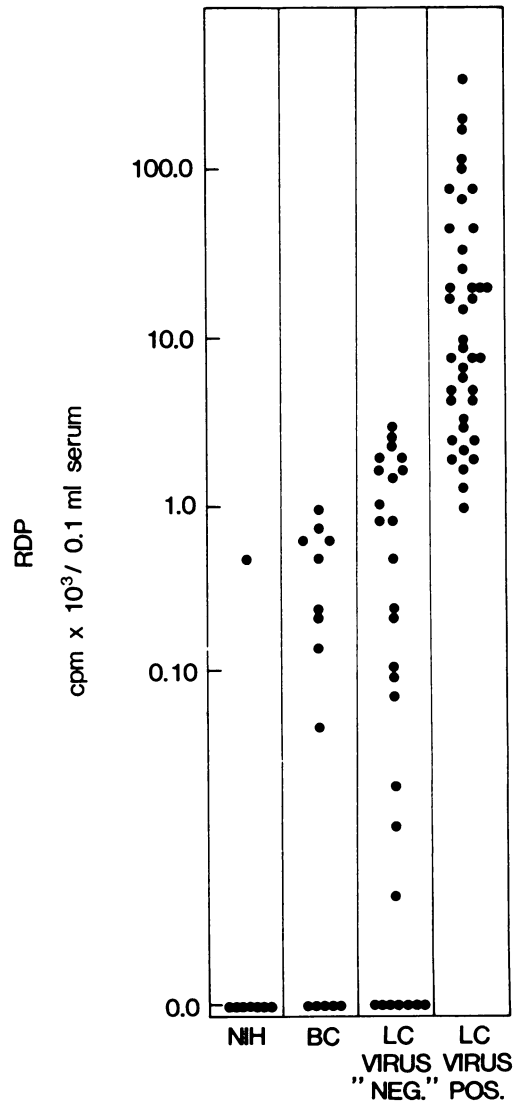

FIG. 2. *Levels of particulate serum RDP in type C virus-positive and -negative mouse populations. RDP was measured as described in the text. Each (●) represents one mouse. LC virus-negative and -positive mice were distinguished by the absence or presence of viremia, respectively, as detected by COMUL assay (5, 6). A few LC mice with borderline elevated RDP counts (1,000 to 5,000 cpm) and negative serum COMUL may have type C virus infections below limits of detectability of the COMUL assay.*

bodies against Rauscher murine leukemia virus polymerase on the serum RDP activity (Table 2). These assays with antibodies were carried out as described previously (11). Particulate serum RDP activity was inhibited by the antibodies in accordance with our previous results with either RDP from disrupted wild-mouse virions or RDP in partially purified form (11, 13). Further characterization of the serum RDP was accomplished by hybridization of the ^{3}H-labeled complementary single-stranded DNA prepared from the endogenous reactions

TABLE 1. *Polymerase activities with oligomer primer-homopolymer templates*

Polymerase	[^{3}H]dTMP incorporation (cpm)		Ratio
	$(dT)_{12-18}$·poly(dA)	$(dT)_{12-18}$·poly(rA)	[$(dT)_{12-18}$·poly(dA)]/[$(dT)_{12-18}$·poly(rA)]
Wild-mouse type C virus strain 292	93	5,377	0.017
LC serum no. 1	56	3,526	0.015
LC serum no. 2	140	7,500	0.018
LC serum no.3	220	8,070	0.027

TABLE 2. *Inhibition of serum RDP activity by antibodies against murine leukemia virus polymerase*

Polymerase	Antibodies (μg)	[^{3}H]dTMP incorporation (cpm)	Inhibition (%)
Wild-mouse type C virus, strain 292	0	45,000	0
	10	9,450	79
LC serum no. 4	0	179,842	0
	10	75,093	58
LC serum no. 5	0	1,807	0
	10	460	75
LC serum no. 6	0	91,461	0
	10	17,461	81

TABLE 3. *Comparison of serum RDP activity with COMUL test for infectious virus and complement fixation or competitive radioimmunoassay for p30 antigen of mouse type C virus*[a]

Source of serum	[^{3}H]dTMP incorporation per 100 μl of serum (cpm)	COMUL[b]		CF[c] spleen	Competitive radioimmunoassay (ng of p30 per 100 μl of serum)
		Tail	Spleen		
Newly trapped normal adult LC mice	5,046	2.0	3.5	+	100
	5,774	3.0	3.5	−	100
	23,209	5.5	3.5	+	170
	7,337	3.5	≥6.5	+	30
	18,311	5.5	5.5	±	95
	113,723	4.5	≥6.5	+	380
	6,648	3.5	3.0	+	34
	235,480	2.5	4.5	±	95
	22,369	3.5	4.5	+	31
	43,344	3.5	4.5	+	67
One litter of 3-week-old LC mice	2,107	0	0	−	<7
	0	0	0	−	<7
	23	0	0	−	<7
	999	0	0	−	<7
	105	0	0	−	<7
	0	0	0	−	<7
	5	0	0	−	<7
	13	0	0	−	<7

[a] Each horizontal column represents an individual serum. RDP was measured as described in the text. COMUL (10), complement fixation (CF; 10), and competitive radioimmunoassays (3), were previously described.

[b] For virus isolation, 0.1 ml of 10% spleen extracts and 20% tail homogenates, frozen at −70°C for several weeks, were assayed undiluted and at 10^{-1} dilution for induction of CF p30 antigen (COMUL test) on mouse SC-1 cells. SC-1 is a cloned wild-mouse embryo cell line which is equally susceptible to N-tropic and B-tropic mouse type C viruses (9). Titers are expressed in $\log_{10}$ mean tissue culture infective dose per milliliter (10).

[c] Aqueous spleen extracts (10%) were tested by CF for mouse p30 antigen using as antisera a 1:20 dilution of pooled sera from Fischer rats bearing Moloney sarcoma virus-induced tumor transplants (10). The specificity of these sera was confirmed by comparison of CF reactions with guinea pig antisera to electrofocus-purified murine luekemia virus p30 antigen (7). A 3 to 4+ reaction was considered positive (+), a 2+ reaction as borderline (±), and a 0 to 1+ reaction as negative (−).

of particulate preparations of two LC virus-positive mouse sera with the ^{32}P-labeled 60 to 70*S* RNA (11) of an LC wild-mouse virus isolate. Hybridization was assayed by the S_1 nuclease method (4). Under the conditions when 57% of the ^{3}H-labeled complementary single-stranded DNA of the wild-mouse type C virus was protected from S_1 nuclease digestion by the 60 to 70*S* RNA of this virus (P. Roy-Burman, unpublished observations), 52 and 48% of the serum ^{3}H-labeled complementary single-stranded DNA products were protected by the viral 60 to 70*S* RNA, respectively, under identical conditions.

The degree of correlation between the particulate RDP and other tests for type C virus (COMUL, complement fixation, and radioimmunoassays) is represented by the data shown in Table 3. It is apparent that the various tests showed a good agreement in a qualitative sense. However, the results failed to show the presence of an absolute quantitative relationship between any two of the assays. This lack of uniform quantitative correlation is not unexpected, as there exists a number of potential variable factors, such as differential in vivo inactivation of virions and their RDP, presence of variable quantities of defective virus particles, complete or partial disruption of the virions in the serum, and the presence of a nonlinear relationship between an enzyme concentration and the amount of product formed (Fig. 1) under a given set of reaction conditions. Moreover, there could be a variety of inhibitory or lysing factors in the serum.

Our results suggest, however, that serum particulate reverse transcriptase assays do provide an additional means of qualitative detection of type C virus infections or spread in the LC or other similar mouse populations. Perhaps, under controlled experimental conditions the assay could be meaningful in rough quantitation of in vivo alteration of virus counts in the animals under study.

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