

# Specific Inhibition of Mammalian Ribonucleic Acid C-Type Virus Deoxyribonucleic Acid Polymerases by Rat Antisera

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Inhibition of the ribonucleic acid (RNA)- and deoxyribonucleic acid (DNA)-dependent DNA polymerase activities of mammalian C-type viruses was obtained with sera from rats bearing murine leukemia virus-induced transplant tumors. Polymerase activities of nonmammalian (viper) C-type virus and murine mammary tumor virus were not inhibited by such sera nor by serum from a rat immunized with the DNA polymerase of feline leukemia virus purified by isoelectric focusing. The latter serum appeared to inhibit preferentially the DNA-dependent DNA polymerase activity of mammalian C-type viruses showing no inhibition of RNA-dependent DNA synthesis.

Recently evidence has been presented which indicates that the ribonucleic acid (RNA)-dependent deoxyribonucleic acid (DNA) polymerases (2, 8) of mammalian C-type viruses are antigenically similar (1). We have been carrying out similar experiments with sera from tumor-bearing animals and a single serum from a rat immunized with a feline C-type virus-derived polymerase. The latter enzyme was isolated by the isoelectric focusing technique by utilizing the alternating copolymer poly d(A-T) as a template.

For screening purposes, sera were generally tested at not less than a 1:50 (v/v) dilution in the reaction mixture to avoid nonspecific stimulatory or inhibitory effects. Although approximately 20% of sera from rats bearing a transplantable lymphosarcoma induced by AKR virus showed at least 50% inhibition of polymerase activity at the test dilution, one serum (obtained from R. Wilsnack, Huntington Research Laboratory, Baltimore, Md.) appeared especially potent, giving significant inhibition of RNA-dependent DNA polymerase activity at dilutions as high as 1:1,000 (Fig. 1). This particular serum also inhibited DNA-dependent DNA polymerase activity assayed by using poly d(A-T) as template and RNA-dependent activity stimulated by exogenous homologous viral 70S RNA (Table 1). The antiserum also inhibited the activity of other mammalian C-type virus polymerases but not that of the mouse mammary tumor virus or the viper C-type virus (Table 2).

Antiserum to the feline virus DNA-dependent DNA polymerase was prepared in a rat by using enzyme purified by isoelectric focusing. The peak of enzyme activity focused at pH 6.5. The peak fractions showed no absorption at 280 nm and contained less than 1 to 2% of the radioactivity recovered from <sup>14</sup>C-amino acid-labeled disrupted virus. At the time of these separations, we did not find RNA-dependent activity in the pH 6.5 fractions when using poly rA:rU as a template; however, with the more sensitive template rA:oligo dT (Collaborative Research, Waltham, Mass.), more recent experiments show an overlap in RNA- and DNA-dependent polymerase activities with no clear evidence of separation. One of three rats immunized with the pH 6.5 fraction produced antibody which inhibited DNA-dependent activity of the feline and other mammalian C-type viruses but not that of mouse mammary tumor virus of the viper C-type virus (Table 3). The remaining two animals did not respond to the immunization procedure. The reactive serum has also not inhibited the DNA polymerase activity of normal feline cells (Table 3; M. Hatanaka, *in preparation*), further attesting to the virus-related nature of the polymerases.

The immune serum was developed after five inoculations with purified enzyme which probably consisted of no more than 10 μg of total protein. In complement fixation (CF) and gel diffusion assays, the serum did not react with the feline C-type virus gs antigen. The gs antigen has an

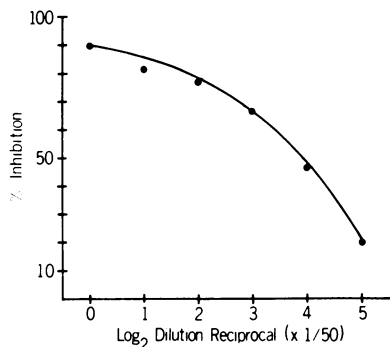


FIG. 1. Titration of anti-polymerase antibody in a rat bearing an AKR virus-induced lymphosarcoma. Assay mixtures contained 0.01 ml of enzyme (Rauscher leukemia virus disrupted by 0.1% Nonidet P-40), 0.01 ml of diluted serum, 0.01 ml of reaction mixture prepared as follows: 5 ml of 1 M glycine-NaOH buffer (pH 8.3), 0.1 ml of 1 M MnCl<sub>2</sub>, 3.75 ml of 4 M NaCl, 2.0 ml of 1 M DTT, adjusted to 20 ml with distilled water, 0.01 ml of poly rA:oligo dT (25 µg/ml), and 0.01 ml of <sup>3</sup>H-deoxythymidine triphosphate (50 µM, 1 Ci/mmole). Serum and enzyme were incubated at 37 C for 30 min followed by addition of the remaining components of the mixture. The complete mixture was then incubated at 37 C for 30 min before addition of 10% cold trichloroacetic acid. Enzyme activity in the absence of serum was used as the 100% activity value. This value did not differ significantly from that obtained in the presence of normal serum.

isoelectric point of 8.3 (5) and is thus clearly distinct from the polymerase. This extends the previous observations of the inability of specific anti-gs sera to inhibit the polymerase activity (1, 3). CF antigen titers of purified, disrupted virus and the purified polymerase and gs antigen fractions of a single isoelectric focus separation were obtained by using four units (based on titration with the respective purified antigens) of anti-polymerase and anti-gs sera. The gs antigen titers were approximately 50-fold higher than polymerase titers in the crude virus preparations and the relative titers of both antigens (peak fractions) from the separation remained in this ratio. Thus, if the gs antigen represents 30% of the total viral protein (4), the polymerase accounts for about 0.6% of the viral protein. This assumes that the CF reactions were with the polymerase and thus could represent an upper limit.

The one peculiarity of this serum which distinguishes it thus far from the serum from tumor-bearing animals and that prepared in rabbits against partially purified enzyme (1) has been its consistent failure to inhibit RNA-dependent DNA polymerase activity by using poly rA:rU or rA:oligo dT as templates (Table 3). Thus,

TABLE 1. Inhibition of RNA- and DNA-dependent DNA polymerase activity by an AKR tumor-bearing rat serum<sup>a</sup>

Test serum	Test template (counts/min)		
	d(A-T)	rA:oligo dT	<sup>70S</sup> RLV RNA
Normal rat . . . . .	1,854	15,407	445
Tumored rat . . . . .	467	695	0

<sup>a</sup> Sera were diluted 1:50 and incubated with disrupted Rauscher leukemia virus (RLV) as described in Fig. 1. The concentration of Nonidet P-40 used, 0.1%, inactivates the endogenous RNA template. Conditions for rAdT were as described in Fig. 1; dAT was used at 10 optical density units at 260 nm per ml, and approximately 0.1 µg of 70S viral RNA was contained in the final mixture. In the incubations with 70S RNA, all four deoxynucleoside triphosphates were present at a final concentration of 12.5 µM, including <sup>3</sup>H-deoxythymidine triphosphate.

TABLE 2. Specificity of polymerase inhibition by tumor-bearing rat sera<sup>a</sup>

Enzyme source <sup>b</sup>	Enzyme activity (counts/min)	
	Normal serum	Immune serum
Mouse:		
RLV . . . . .	4,000	460
H-MSV . . . . .	48,000	1,500
Rat:		
MSV(RaLV) . . . . .	13,000	4,300
Hamster:		
HaLV . . . . .	22,000	12,000
Viper . . . . .	5,300	4,400
Mouse:		
MTV . . . . .	12,000	11,000

<sup>a</sup> Sera were used at a 1:50 dilution as described in Fig. 1, with rA:dT as template.

<sup>b</sup> RLV, Rauscher leukemia virus; H-MSV, Harvey strain of mouse sarcoma virus; RaLV, rat leukemia virus; HaLV, hamster leukemia virus; MTV, mammary tumor virus.

primarily because of inability to separate physically RNA- and DNA-dependent activities, we suggest that the polymerase(s) may be a complex molecule, e.g., multiple subunits, with distinct antigenic sites. The single immunized rat may have preferentially produced antibody to one site at or near the DNA-dependent catalytic site. The possibility of physically separable molecules has, however, been raised in recent studies of Rous sarcoma virus (6).

In summary, our data agree with the previous finding that the RNA- and DNA-dependent

TABLE 3. *Specificity of rat anti-feline leukemia virus polymerase serum*

Virus	Test template <sup>a</sup>	
	Poly d(A-T)	rA:oligo dT
Feline leukemia virus.....	1,117/7,213 (85) <sup>b</sup>	10,800/11,500 (6)
AKR.....	886/4,703 (81)	14,000/12,500 (0)
Hamster leukemia virus.....	162/983 (84)	7,742/8,900 (13)
Rauscher leukemia virus.....	818/3,296 (75)	21,322/24,600 (13)
Viper C-type.....	6,215/6,400 (3)	NT
Mouse mammary tumor virus.....	4,774/4,520 (0)	NT
Feline cell polymerase.....	3,045/2,675 (0)	NT

<sup>a</sup> Sera at a final dilution of 1:100 were incubated with viruses disrupted by 0.1% Nonidet P-40 as described in Table 1 and Fig. 1 with d(A-T) and rA:oligo dT as templates and <sup>3</sup>H-thymidine triphosphate as substrate. The normal tissue DNA polymerase preparations used were obtained from several virus-free cell lines as well as extracts of cat tissues. No inhibition was obtained with any of these preparations. The data given are for a feline tongue cell line. NT = not tested.

<sup>b</sup> Enzyme activity (counts/minute): immune serum/preimmune serum (per cent inhibition).

polymerases of mammalian C-type viruses share similar antigenic sites (1). Since normal cell polymerase activity is not inhibited by the available antisera, a method for distinguishing virus-specific enzymes in infected or transformed cells is provided. Similar conclusions based on detailed studies have recently been reported (7).

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