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Immunological Relationships of Reverse Transcriptases from Ribonucleic Acid Tumor Viruses

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Antiserum to partially purified reverse transcriptase from the Schmidt-Ruppin strain of Rous sarcoma virus has been prepared and characterized. Antibody to the avian polymerase inhibited the reverse transcriptase activity of avian C-type viruses but had no effect on the polymerase activity from C-type viruses of other classes. The known mammalian C-type viral polymerases were significantly inhibited only by the antiserum to murine C-type viral polymerases; reverse transcriptases from four other mammalian viruses were immunologically distinct from both avian and mammalian C-type viral polymerases. Partially purified murine leukemia viral DNA polymerase activity was comparably reduced by specific antibody regardless of the template used for enzyme detection.

Antibody to the deoxyribonucleic acid (DNA) polymerase activities of murine C-type viruses has been previously demonstrated in the sera of rats with murine leukemia virus (MuLV)-releasing tumors and in the sera of rabbits immunized with partially purified murine viral polymerases (2). These studies suggested that the reverse transcriptases of mammalian C-type viruses were immunologically related. In the present report, we describe the preparation of a rabbit antiserum against a partially purified avian C-type viral reverse transcriptase and extend our previous studies of the immunological relationships between viral reverse transcriptases.

MATERIALS AND METHODS

Viruses. The sources of several of the viruses used in the present studies have been detailed previously (16); they were purified by sucrose density gradient sedimentation and were used as 100- to 300-fold concentrates unless otherwise specified. Rous-associated viruses types 1 and 2 (RAV-1 and RAV-2) and the Bryan strain of Rous sarcoma virus (B-RSV) were obtained from the Viral Resources and Logistics Segment, National Cancer Institute, National Institutes of Health (NIH), Bethesda, Md. The C-type virus of ophidian origin (viper virus) originally described by Zeigal and Clark (17) was grown in a viper spleen cell line and kindly provided by R. Gilden (Flow Laboratories, Rockville, Md.). A nontransforming "helper" leukemia virus (RaLV) was isolated from a stock of the rat-tropic sarcoma virus, MSV-0 (1). The rat mammary carcinoma cell line R-35, producing a C-type virus (4), was kindly supplied by M. Ahmed (Pfizer, Maywood, N.J.). Visna virus was grown in a line of sheep testes cells and kindly provided by K. K. Takemoto and L. B. Stone (NIH, Bethesda, Md.). Mason-Pfizer monkey virus (MP-MV; reference 9) was grown in either monkey embryo cells or a human lymphoblastoid line (NC-37); some MP-MV preparations were provided by M. Ahmed. The primate syncytium-forming ("foamy") virus type 3 was propagated in monkey kidney cells or rat cells (13).

Polymerase purification. DNA polymerase from the Rauscher strain of murine leukemia virus (R-MuLV), the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV), and the MP-MV were partially purified by gel filtration and phosphocellulose chromatography as reported earlier, except that the dextran-polyethylene glycol step was omitted (14).

Preparation of antisera. New Zealand white rabbits (2 to 3 kg) were inoculated with 1 to 2 ml of concentrated, partially purified viral polymerase (<5 μg of protein) emulsified with an equal volume of complete Freund's adjuvant. Inoculation was either into the hind footpads or at multiple subcutaneous sites. Booster doses were administered subcutaneously at subsequent 2- to 3-week intervals. Control rabbit sera consisted of either preimmunization bleedings from the same rabbits or sera from comparable nonimmunized rabbits.

Chromatographic procedures. High-titered antisera were fractionated by precipitation with 50% ammonium sulfate, and the immunoglobulin G (IgG) was purified by diethylaminoethyl (DEAE) chromatography as previously described (2). The immunoglobulin fractions were concentrated by ultrafiltration with a P-30 membrane (Amicon, Lexington, Mass.), and protein concentrations were determined by the procedure of Lowry et al. (10).

Polymerase assays. Each reaction mixture was incubated at 37 C for periods of time (30 to 60 min) when the reaction was still linear and contained in 0.10 or 0.20 ml, as indicated in the appropriate legends: 0.04 M tris(hydroxymethyl)aminomethane-hydrochloride (*p*H 7.8); 0.06 M potassium chloride; 0.002 M dithiothreitol; 2×10^{-5} M ^{3}H-thymidine triphosphate (5,000 counts per min per pmole); and, unless otherwise noted, polyriboadenylic oligodeoxythymidylic acid (poly rA·oligo $dT_{(12-18)}$; Collaborative Research, Waltham, Mass.; references 12–17). All templates were included at saturating levels: 0.02 absorbancy unit at 260 nm per 0.1 or 0.2 ml. For reactions containing poly rA·oligo dT or polyriboadenylic·polyribouridylic (poly rA·poly rU), the divalent cation used was manganese (10^{-4} M manganese acetate). In reactions containing "activated" DNA (14) or viral ribonucleic acid (RNA), the divalent cation used was magnesium (6×10^{-3} M magnesium acetate); 5×10^{-4} M unlabeled deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxyguanosine triphosphate were also included. Incorporation in the absence of added templates was between 0.1 and 0.2 pmoles and was subtracted where indicated. The sources of isotopes, polymers, and the preparation of enzymes have been previously described (14); enzymes referred to as partially purified represent the Sephadex G-100 or phosphocellulose fractions as indicated in appropriate legends. Murine viral polymerase partially purified by phosphocellulose chromatography does not have detectable nuclease activity nor group-specific (gs) antigens; both nuclease and gs antigen reactivity are recovered in the column void volume (*unpublished data*). MuLV RNA was the gift of M. Hatanaka (Flow Laboratories, Rockville, Md.).

RESULTS

Antiserum inhibition of avian and murine polymerase. Sera from animals inoculated with polymerase preparations first demonstrated inhibition of viral enzyme activity 8 to 12 weeks after primary immunization. Once an antibody response was detected, animals were given additional inoculations until antiserum titers reached maximal levels. As shown in Fig. 1A, 19 μg of R-MuLV polymerase antisera reduced R-MuLV polymerase activity by more than 50% of the control incorporation, whereas SR-RSV polymerase was unaffected by as much as 162 μg of serum protein. Conversely, antiserum to the SR-RSV polymerase inhibited partially purified SR-RSV enzyme over 90% but had no effect on the R-MuLV polymerase activity (Fig. 1B).

Purified IgG isolated by DEAE chromatography from immune sera showed the same patterns of inhibition as did whole serum but had a higher specific activity for enzyme inhibition. Whereas 50 to 100 μg of whole serum protein per reaction was required for 90% inhibition of R-MuLV polymerase activity (Fig. 2A), less than 10 μg of IgG protein gave comparable inhibition (Fig. 2B). Although stimulation of viral polymerase activity was frequently observed with whole serum at low concentrations (10 to 50 μg per reaction mixture; reference 2), inhibition of viral polymerase by control sera or DEAE-purified IgG fractions was observed at high protein levels (>200 μg per reaction mixture). This

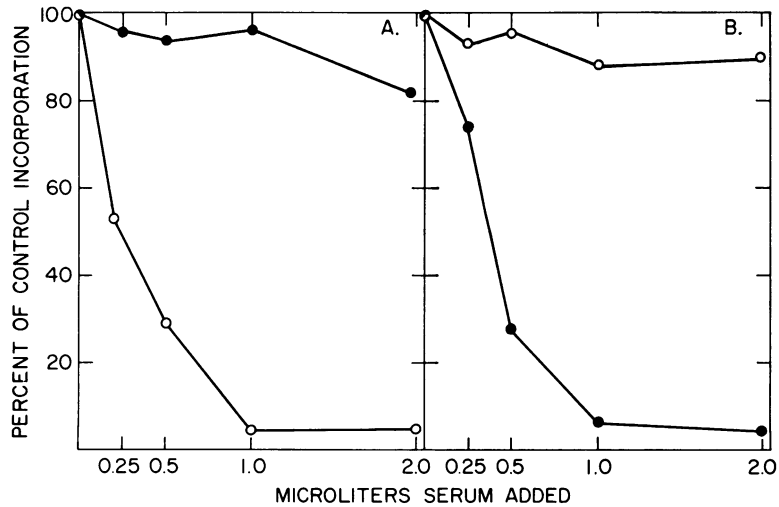

FIG. 1. *Inhibition of partially purified viral polymerase by immune whole sera. The effect of anti-R-MuLV polymerase whole sera (76 mg/ml) on ^{3}H-thymidine monophosphate incorporation with phosphocellulose-purified R-MuLV polymerase (3 μg, ○) and phosphocellulose-purified SR-RSV (5 μg, ●) is shown in Fig. 1A. The effect of anti-SR-RSV whole sera (66 mg/ml) on R-MuLV and SR-RSV polymerase activity is shown in Fig. 1B. Incorporation in the absence of serum was 4.2 and 3.8 pmoles for R-MuLV and SR-RSV, respectively.*

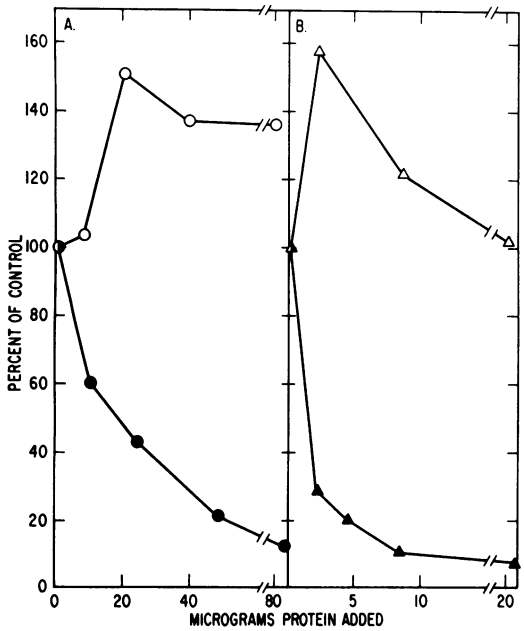

FIG. 2. *Purification of polymerase antibodies by DEAE fractionation. Figure 2A shows the effect of control (○) and immune anti-R-MuLV serum (●) on R-MuLV polymerase activity (5 μg). After DEAE chromatographic purification, the effects of immue (▲) and control serum IgG (△) on phosphocellulose-purified R-MuLV polymerase are shown in Fig. 2B.*

inhibition may be due to contaminating nucleases or proteases in the preparation.

Cross-inhibition studies. To characterize further the cross-reactivity of antibodies to viral reverse transcriptases, the effect of the avian and murine C-type polymerase antisera on the DNA polymerase activities of other RNA-containing viruses was studied. IgG from antiserum to the SR-RSV polymerase inhibited approximately matched enzyme activities from the disrupted virions of B-RSV, RAV-1, and RAV-2 and paritally purified polymerase from avian myeloblastosis virus to approximately the same degree as noted with partially purified SR-RSV polymerase. However, none of these avian viral enzymes was inhibited by IgG from anti-R-MuLV polymerase sera. Conversely, antiserum to the SR-RSV polymerase failed to inhibit significantly the viper C-type viral polymerase or any mammalian C-type viral enzymes. Previous studies have also reported inhibition of the hamster C-type viral polymerase with similar antisera (2). Under conditions where there was more than 90% inhibition of the R-MuLV enzyme, the inhibition of the rat leukemia and feline leukemia polymerases was 65 and 45%, respectively, at comparable levels of incorporation. In this and other experiments not shown, antiserum to R-MuLV enzyme was consistently more potent against the homologous enzyme than against rat, hamster, or

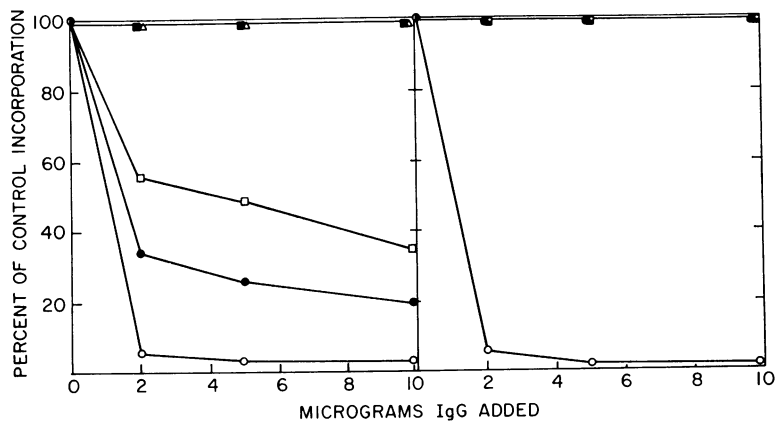

FIG. 3. *Immunological relationships of C-type and other mammalian viral reverse transcriptases. Figure 3A shows the effect of anti-R-MuLV polymerase sera on ^{3}H-thymidine monophosphate incorporation by the viper virus (72 μg, ■), SR-RSV (1.5 μg, △), FeLV (2.6 μg, □), RaLV (86 μg, ●), and R-MuLV (1.3 μg, ○). Control serum IgG was tested at comparable concentrations, and the level of incorporation at each point was considered to represent 100% incorporation. Figure 3B indicates the effect of the same antibody preparation on visna (82 μg, ●), MP-MV (1.5 μg, □), simian "foamy" virus (34 μg, ■), and R-MuLV (1.4 μg, ○) polymerase activities. Incorporation with these viruses ranged between 4.1 and 96.7 pmoles in the presence of control sera IgG. The SR-RSV, R-MuLV, feline leukemia virus, and MP-MV polymerases were partially purified preparations; the others were from disrupted virions.*

feline C-type polymerases. This suggests the reverse transcriptases of mammalian C-type virus, although immunologically related, are not identical.

Whereas antibody to R-MuLV polymerase inhibited R-MuLV polymerase by over 90%, it failed to inhibit visna, MP-MV, or simian "foamy" virus type 3 viral enzymes from disrupted virions at comparable levels of DNA synthesis (4.1 to 96.7 pmoles; Fig. 3B). Murine mammary tumor virus (MTV) polymerase activity was not affected by either the avian or murine antiserum. Since antisera to the R-MuLV reverse transcriptase were equally potent whether tested against disrupted whole virus or partially purified enzyme, lack of inhibition of the reverse transcriptases of visna, MTV, or simian "foamy" virus type 3 probably cannot be attributed to inaccessibility of the enzyme in disrupted virion preparations. In the case of the MP-MV reverse transcriptase, no inhibition by antibody was observed with either partially purified enzyme or disrupted virion preparations.

In other studies not shown, the effect of cells employed for viral growth was examined. MP-MV grown in human lymphoid cells (NC-37) was not inhibited by the anti-murine polymerase sera, although R-MuLV propagated in human kidney cells could be neutralized by greater than 90%. Simian "foamy" virus grown in rat cells employed for the growth of several strains of MuLV and RaLV was also not inhibited by the R-MuLV polymerase antisera.

Inhibition of polymerase with different templates. Table 1 shows the requirements of viral RNA as a template for the partially purified murine viral polymerase. The reaction required all four deoxyribonucleotide triphosphates and was inhibited by ribonuclease A. The reaction was stimulated over 100-fold by the addition of "activated" DNA. This latter activity was not sensitive to ribonuclease A.

TABLE 1. *Conditions for viral RNA stimulation of partially purified MuLV reverse transcriptase*

Condition[a]	Amt (pmoles) incorporated
Complete	0.58
Minus viral RNA	0.07
Minus deoxyadenosine triphosphate	0.07
Plus ribonuclease A (10 μg/ml)	0.07
Plus activated DNA	68.2
Plus activated DNA + ribonuclease A (10 μg/ml)	69.8

[a] Reaction mixtures were incubated for 60 min at 37 C and are as described in Materials and Methods in 0.20 ml and contained 5.0 μg of Sephadex G-100-purified viral enzyme and MuLV viral RNA.

TABLE 2. *Inhibition by antiserum of MuLV polymerase activity assayed with different templates*[a]

Template[a]	Serum added			
	None (Δ pmoles incorporated)	Prebleed (Δ pmoles incorporated)	Antiserum (Δ pmoles incorporated)	Per cent inhibition
MuLV RNA	0.4	0.3	0.02	95
"Activated" DNA	74.4	63.1	3.3	96
Poly rA·oligo dT	395.6	464.7	27.0	93
Poly rA·poly rU	61.5	57.2	2.1	97

[a] Polymerase assays are as described in Materials and Methods for 0.20-ml reaction mixtures. Each reaction mixture contained 5.0 μg of purified globulin protein. In the experiment with poly rA·oligo dT and poly rA·poly rU, 10^{-4} M manganese acetate replaced magnesium acetate as the source of divalent cation.

Our antibody inhibition studies have been performed with poly rA·oligo dT as the template because of its great sensitivity with the viral enzyme. However, as shown in Table 2, IgG from antiserum to R-MuLV polymerase inhibited the activity of R-MuLV enzyme with each class of templates utilized by the enzyme, including viral RNA, "activated" double-stranded DNA, synthetic double-stranded RNA, and RNA-DNA hybrids. Comparable levels of inhibition (93 to 97%) were noted with each class of template. These data might support previous results which suggested that both the RNA-dependent and DNA-dependent DNA polymerase activities are associated with a single enzyme (5, 14).

Relationships between viral polymerases. Table 3 summarizes our studies of enzyme inhibition by antisera to the murine and avian C-type viral polymerases. As previously noted, representatives of all mammalian C-type viruses were inhibited, including a C-type rat virus, R-35, associated with a line of rat mammary adenocarcinoma cells (4). All avian viral polymerases tested were comparably inhibited by the antiserum to SR-RSV polymerase. However, there exists a relatively large group of viruses whose reverse transcriptases are not significantly (>10%) inhibited by the antisera presently available; these viruses include the viper C-type virus, murine MTV, visna, MP-MV, and simian "foamy" virus.

TABLE 3. *Cross-reactions between DNA polymerases of different viruses and antipolymerase sera*[a]

Viral polymerases inhibited by		
Anti-MuLV polymerase	Anti-AvLV polymerase	Neither
MuLV	SR-RSV	Viper
RaLV	B-RSV	Murine MTV
Rat R-35	RAV-1	Visna
HaLV	RAV-2	MP-MV
FeLV	AMV	Simian "foamy"

[a] Assays were as described in Materials and Methods. MuLV, SR-RSV, and MP-MV were tested as partially purified preparations; other polymerases were from disrupted virions. Levels of incorporation in the absence of serum ranged from 3.3 to 98.6 pmoles of ^{3}H-thymidine monophosphate (TMP). Antibody preparations were tested at (IgG) levels of 1 to 20 μg of protein per reaction. Sera were regarded as negative unless there was greater than 10% reduction in ^{3}H-TMP incorporation compared to incorporation in the presence of comparable levels of control IgG. HaLV, hamster leukemia virus; FeLV, feline leukemia virus.

DISCUSSION

An antibody to the SR-RSV reverse transcriptase has been prepared by immunizing rabbits with partially purified enzyme preparations. This antiserum is capable of inhibiting the viral enzymes of other avian C-type tumor viruses as well as that of SR-RSV. In combination with antisera prepared against murine viral reverse transcriptases (2), it has been possible to extend the initial observations concerning the immunological cross-reactivity of viral enzymes. Although antibodies against the avian and murine C-type viral enzymes inhibit polymerase activities from C-type viruses of the homologous classes, neither inhibit the polymerases of other classes. These results clearly demonstrate that the avian and murine C-type viral reverse transcriptases are immunologically distinct.

The viral reverse transcriptases are distinguished from the presently recognized internal core proteins by several features. Molecular weights of $\geq$110,000 and 70,000 reported for avian (5) and murine polymerases (15), respectively, are significantly larger than reported values (molecular weight 12,000 to 35,000) for the major internal proteins, the "group-specific" or gs antigens (3, 7). Monospecific antisera against purified murine gs antigens do not inhibit murine polymerase (8), and there is no correlation between serum anti-gs titers and anti-polymerase activity (2). The major internal structural protein (molecular weight ~30,000) of murine C-type viruses contains an antigenic determinant common to the other mammalian C-type viruses (6) and consequently is an interspecies antigenic determinant. Since the DNA polymerase activities of murine, feline, rat, and hamster C-type viruses were each inhibited by the anti-MuLV polymerase sera, reverse transcriptases represent a second interspecies antigen of mammalian C-type viruses. The avian polymerase also has antigenic specificities common to the various avian leukosis and sarcoma viruses. Whether the antiserum to SR-RSV viral polymerase will inhibit avian C-type viral polymerases from species other than chickens is not known.

Antiserum to avian and mamalian C-type viral polymerases does not inhibit the viral enzymes of murine MTV, visna, MP-MV, or "foamy" virus. This suggests that there are other distinct groups of mammalian viruses with reverse transcriptases, a conclusion supported by a variety of morphological, immunological, and biological data (11, 12, 13, 16). Consequently, sera prepared against C-type viral polymerases may be a useful adjunct in viral classification. For example, the virus released by the rat tumor line R-35 was inhibited by antibody to the MuLV enzyme. Therefore, in addition to its morphological resemblance to C-type rather than B-type particles (4), immunologically it also appears to be a member of the C-type virus group.

Comparable levels of antibody inhibition of the murine viral enzyme was observed with all classes of templates tested. Therefore, an unclassified DNA polymerase activity can be immunologically identified as viral with any class of template as long as the polymerase is immunologically related to a known viral reverse transcriptase. In the case of C-type viruses, such reagents (2, 12a) are now available. The cross-reactivity of C-type RNA-containing viral polymerases from four mammalian species suggest that other mammalian C-type viral polymerases might also be immunologically related.

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