Reverse Transcriptases of Primate Viruses as Immunological Markers

Abstract. *Antibodies were prepared against the DNA polymerases (reverse transcriptases) of three potentially oncogenic RNA viruses of primates. Two type C viruses, isolated from a woolly monkey fibrosarcoma and from a gibbon ape lymphosarcoma, have polymerases that are immunologically related to each other and are distinct from the type C viruses isolated from other mammals.*

While the RNA-directed DNA polymerases (reverse transcriptases) of several RNA-containing viruses catalyze common biochemical reactions (*1*), the enzymes exhibit major immunological differences (*2*). With the use of antibodies to the viral reverse transcriptases, viruses can be distinguished on the basis of their type and species of origin. Since there are at least five virus groups that contain reverse transcriptases (*2*), an immunological approach is helpful for the identification of unknown viral isolates.

Some virus groups, such as the type C viruses, have been associated with naturally occurring tumors (*3*); others, such as the "foamy" virus group (*4*), are relatively common but have no established pathogenicity. Among the type C viruses, the polymerases of reptilian, avian, and mammalian viruses form immunologically distinct classes. At present, there are four RNA-containing viruses of subhuman primate origin which have a reverse transcriptase: a type C virus called SSV-1 (*5*), isolated from a woolly monkey fibrosarcoma (*6*), a type C virus isolated from a gibbon ape lymphosarcoma (*7*), a virus isolated from a mammary adenocarcinoma of a rhesus monkey, called Mason-Pfizer monkey virus (MP-MV) (*8*), and the syncytium-forming (foamy) viruses which have been isolated from several primate species (*9*). Our studies were undertaken to determine the immunological relations between these viral polymerases and to compare their properties with the enzyme from other mammalian reverse transcriptase–containing viruses, including a type C virus growing in the human cells RD-114 (*10*).

Rabbit immunoglobulin G (IgG) from antiserum prepared against the polymerase of the type C virus from the woolly monkey was tested against the reverse transcriptase from each of the four known primate polymerase-containing viruses (Fig. 1). The woolly monkey virus polymerase was 90 percent inhibited by as little as 30 μg of IgG, whereas 250 μg of IgG from an unimmunized rabbit under identical conditions had no significant (< 10 percent) effect (Fig. 1A). Under identical conditions (Fig. 1B), the gibbon type C viral polymerase was 70 percent inhibited by 30 μg and 90 percent inhibited by 100 μg of IgG; control IgG again failed to inhibit. The reverse transcriptases from the MP-MV and foamy virus type 3 were not significantly affected by the IgG from antiserum to woolly monkey polymerase as compared to IgG from control serums. Thus, antiserum prepared against one primate type C virus (woolly monkey virus) efficiently inhibited another (gibbon virus), but did not inhibit the reverse transcriptases of two other primate viruses.

Rabbit antiserum was also prepared against the MP-MV polymerase (*11*). When tested against MP-MV reverse transcriptase, inhibition of enzyme activity was detected (Fig. 2C); however, 400 μg of IgG was required to achieve 90 percent inhibition. When the MP-MV polymerase antiserum was tested against the other primate viral polymerases, no inhibition relative to control serum was observed. Thus, experiments with IgG from antiserum to woolly monkey polymerase show that the two known primate type C viruses contain polymerases that are immunologically related to each other but distinct from the polymerases of MP-MV and primate syncytium-forming virus type 3. Moreover, studies with the antiserum to MP-MV polymerase suggest that the reverse transcriptases of MP-MV and foamy virus are not closely related. These results are consistent with morphological studies and immunological studies of other virus proteins (*12*), which also indicated that these viruses can be distinguished from one another, as well as from primate type C viruses.

A type C virus, RD-114, has been isolated from a human rhabdomyosarcoma (*10*) after the tumor cells were passaged in the brain of a fetal cat. The major internal polypeptide (gs antigen) of this virus is immunologically distinct from that of various isolates of feline type C viruses (*13*), and earlier studies with its polymerase have shown that the RD-114 polymerase is immunologically distinguishable (*2*) from the polymerase of type C virus of cats, mice, rats, or hamsters. Along with the failure of the primate type C virus polymerases to react with antiserum prepared against the lower mammalian type C polymerases, the failure of the RD-114 enzyme to react with the same antiserums was consistent with the possibility that this virus might be human in origin (*2, 13*). Thus, we set out to determine whether this virus is closely related to the other primate type C viruses. Antiserums to the RD-114 and the gibbon type C viral polymerases were prepared. Antiserum to polymerases from the woolly monkey, the gibbon ape, and RD-114 were tested against the polymerases of the woolly monkey, gibbon, RD-114, cat, and an avian type C virus—avian myeloblastosis virus (*14*) (Table 1). In each case, the end point was taken as that amount of antiserum required for 30 percent inhibition as compared to that of control antiserum. In repeated experiments, less than 10 percent inhibition could not be reliably assessed; more than 30 percent inhibition was not proportional

Table 1. Immunological relatedness of type C viral polymerases. Assays were performed as described in the legend to Fig. 1. The enzymes of the woolly monkey virus, gibbon ape virus, and RD-114 virus were purified on phosphocellulose prior to assay for inhibition; the enzymes of the feline and avian virus were purified by G-100 Sephadex chromatography before use (*18*). As in prior reports (*2*), comparable degrees of enzyme inhibition have been obtained with disrupted virion preparations. Protein was determined by the method of Lowry *et al.* (*19*). Homologous reactions are italicized.

Virus	Immunoglobulin G required for 30 percent inhibition of enzymatic activity (μg)				
	Woolly	Gibbon	RD-114	Feline	Avian
Woolly	*1.0–1.5*	20–30	> 150	> 100	> 200
Gibbon	2.0–2.5	*15–20*	> 150	> 100	> 200
RD-114	20–40	200–300	*5–10*	> 100	> 200
Feline	50–100	200–300	> 150	*5–10*	> 200
Avian	> 150	> 300	> 150	> 100	*5–10*

in a linear fashion to added antiserum. Thus, in each case, the amount (in micrograms) of IgG required for a 30 percent inhibition of a given incorporation is noted. This method of expressing immunological relatedness by defining the amount of antibody required for a given response has been used in studies of protein evolution with microcomplement fixation assays of the lactate dehydrogenases, albumins, and other proteins (*15*). With the antiserum to woolly monkey polymerase, the woolly monkey virus enzyme is 30 percent inhibited by 1.0 to 1.5 μg of IgG. Inhibition of the gibbon ape virus enzyme requires only slightly more IgG. The RD-114 and feline viral polymerases are not comparably inhibited until, respectively, 20 to 40 times and 50 to 100 times more IgG is added. The avian type C virus is not significantly inhibited even after addition of 100 to 150 μg of IgG from antiserum to woolly monkey polymerase.

The IgG from antiserums to gibbon ape and RD-114 polymerases inhibit their respective homologous enzymes; 15 to 20 μg of IgG from antiserum to gibbon polymerase inhibited the polymerase 30 percent, whereas 20 to 30 μg inhibited the woolly monkey polymerase activity. Ten times more antibody was required to inhibit comparably the RD-114 and feline type C polymerases. The IgG from rabbit antiserum to RD-114 inhibited only its homologous enzyme, and failed to inhibit either of the two known primate type C polymerases or the feline type C polymerase.

The results indicate that the reverse transcriptases of two primate type C viruses are immunologically closely related, despite the fact that the viruses themselves are not from closely related primates; one is from a South American woolly monkey and the other is from an Asiatic gibbon ape. With both antiserums to the polymerases the homologous enzyme is more efficiently inhibited, but comparable inhibition is attained with less than twice the amount of IgG in the heterologous system (woolly monkey polymerase antiserum tested against gibbon polymerase and vice versa).

As is consistent with earlier results (*2*), the antiserums to woolly monkey and RD-114 polymerases distinguish immunologically the RD-114 polymerase from the polymerase of known cat type C viruses. However, our results indicate that the RD-114 polymerase is not as closely related to either of the two known primate type C viruses as

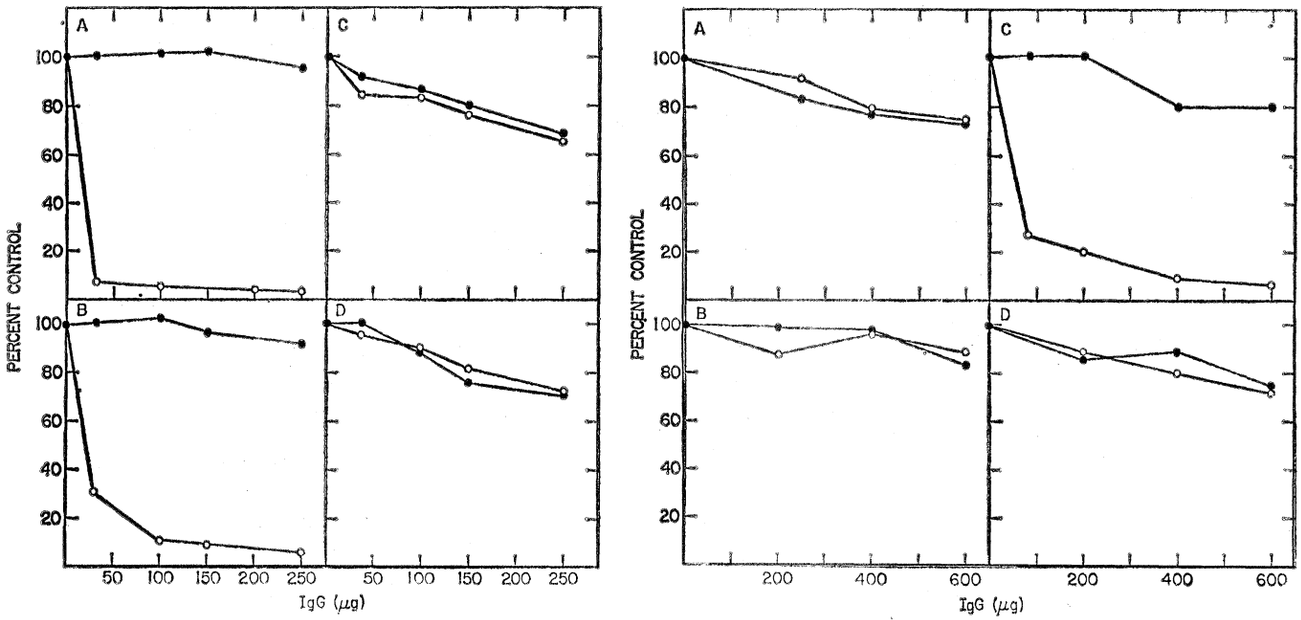

Fig. 1 (left). The type C virus from a gibbon ape was grown on a strain of normal human diploid embryo fibroblasts, M-413. The woolly monkey type C virus was grown in either M-413 or in a clone of murine sarcoma virus–transformed rat cells, KNRK clone 32 (*17*); primate virus grown in this cell line has remained free of detectable mouse or rat type C virus or mouse or rat gs antigen (*16*). The Rickard and Theilin strains of feline leukemia virus were obtained from University Laboratories (Highland Park, N.J.). The RD-114 virus was grown in either the original human rhabdomyosarcoma cell line, RD-114, or in M-413. The Mason-Pfizer monkey virus (MP-MV) was grown in either monkey embryo cells or a human lymphoblastoid line, NC-37. The primate syncytium-forming (foamy) virus type 3 was propagated in secondary African green monkey kidney cells. As was reported with murine and feline type C viruses (*2*), in each instance where a virus was grown in two or more cell types, the antigenic properties of the polymerase were the same regardless of the cells in which the virus was grown. The viral DNA polymerases were assayed as described previously with a few modifications (*2, 18*). Each 0.05-ml reaction mixture was incubated for 60 minutes at 37°C. Enzyme preparations and serums were first incubated for 5 minutes at 24°C in siliconized tubes in 0.04-ml reaction mixtures containing 0.02 percent Triton X-100, 0.05*M* tris-HCl (*p*H 7.8), 0.075*M* potassium chloride, 0.0025*M* dithiothreitol, and 5×10^{-4}*M* manganese chloride. The reaction was then initiated by addition of 0.02 A_{260} units of $(rA)_n \cdot (dT)_{12-18}$, a copolymer of polyadenylate and oligothymidylate as indicated, and 2×10^{-5}*M* [^{3}H]thymidine triphosphate (6000 count/min per picomole). The addition of the Triton X-100 and the preliminary incubation with enzymes and antiserums prior to additions of polymer and substrate minimized nonspecific stimulation of the reaction by control serum protein preparations. All reactions were performed with partially purified enzyme (*18*) under conditions where the enzyme is limiting and where incorporation ranged from 4 to 6 pmole; values for the incorporation without enzyme were 0.1 to 0.2 pmole. ●——●, Control serum; ○——○, antiserum to woolly monkey polymerase. (A) Woolly monkey viral polymerase. (B) Gibbon ape viral polymerase. (C) Mason-Pfizer monkey viral polymerase. (D) Primate syncytium-forming (foamy) type 3 viral polymerase. Fig. 2 (right). Assays were performed as indicated in the legend to Fig. 1, except that the antibody preparation was the IgG fraction of serum from a rabbit immunized with the MP-MV polymerase.

these viruses are related to one another. Similar data have also been obtained by comparing the gs proteins of woolly monkey, gibbon, and RD-114 type C viruses (*16*). Although RD-114 satisfies the morphological and immunological criteria for classification as a type C virus (*13*), it is not yet possible to assign its species of origin. Additional isolates from other primates, including man, will be necessary to establish the generality of a primate type C virus group based on the antigenic properties of the reverse transcriptase.

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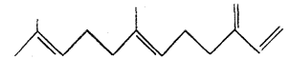
Aphid Alarm Pheromone: Isolation, Identification, Synthesis

Abstract. *A broadly interspecific aphid alarm pheromone was isolated from several economically important species of aphids and identified as* trans-*β-farnesene.*

Aphids are repelled by the odor of droplets released from their cornicles (*1*). When an aphid is attacked by an insect predator, these droplets are produced causing nearby aphids to escape or drop from their feeding sites on plants (*2*). Such a repellant substance is therefore defined as an alarm pheromone (*3*).

Aphids were homogenized in a Ten Broeck tissue homogenizer in a mixture of diethyl ether and methanol (3 : 1, by volume), the homogenate was filtered, and the filtrate was dried over anhydrous sodium sulfate. After evaporation of the solvent in vacuo, the residue was dissolved in hexane and chromatographed on a Florisil column. Only the hexane eluate (hydrocarbon fraction) showed biological activity. The hydrocarbons were further separated by column chromatography over Florisil impregnated with silver nitrate, and eluted with increasing concentrations of diethyl ether in hexane. Biological activity was recovered in the fraction eluted with 6 percent ether in hexane. Comparison of the chromatographic mobility of the active material with that of multiply unsaturated hydrocarbon standards indicated the presence of a compound containing three to four olefinic double bonds. The active fraction displayed two prominent peaks by gas-liquid chromatographic analysis on Carbowax 20-M. Biological activity was recovered from the gas chromatograph by trapping the effluent in capillaries chilled in Dry Ice. One peak contained the alarm pheromone, which was then examined by combined gas chromatography–mass spectrometry (*4*). The pheromone had a molecular ion of 204, which, on the basis of an entirely hydrocarbon composition, was equivalent to the molecular formula $C_{15}H_{24}$ with four degrees of unsaturation, and which, in turn, suggested a farnesene-type hydrocarbon. *trans,trans-α*-Farnesene has been identified from Dufour's gland in ants (*5*). To investigate the possibility of a similar compound in aphids, the more readily prepared *trans-β*-farnesene was synthesized by heating 10 g of nerolidol in 100 ml of dimethyl sulfoxide in a glass-lined reaction bomb at 150°C for 20 hours (*6*). The reaction yielded 74 percent of hydrocarbon products, from which *trans-β*-farnesene was isolated by preparative gas chromatography. The structure was authenticated by comparison of its infrared, ultraviolet, and nuclear magnetic resonance spectra with reported values (*7*).



trans-β-Farnesene

The retention times on apolar (SE-30, XE-60) and polar phases (Carbowax 20-M) in gas-chromatographic analysis were identical to those for the aphid alarm pheromone.

The mass spectra of synthetic *trans-β*-farnesene and the aphid alarm pheromone were identical not only in the intensities of all the ions, but also with respect to the shapes and intensities of the metastable ions, especially those at *m/e* (mass to charge) 24.3, 37.1, 63.8, 89.0, and 127.0.

Further proof of identity was obtained by treating portions of aphid pheromone and the synthetic *trans-β*-farnesene with one molar equivalent of *m*-chloroperoxybenzoic acid in dichloromethane solution. Both reacted to give an epoxide with identical retention times on polar and apolar phases in gas chromatography. The mass spectra reveal a monoepoxide (parent ion *m/e* 220) in which the intensity of all the ions, and especially the metastable ions at *m/e* 63.1, 63.9, 77.2, and 88.8, are identical in shape and intensity.

Finally, biological assay of the synthetic *trans-β*-farnesene against ten species of aphids indicated complete biological activity (*8*).

trans-β-Farnesene was identified as the alarm pheromone by the above