COMPARISON OF RNA-DIRECTED DNA POLYMERASES FROM XENOTROPIC AND ECOTROPIC VIRUSES*

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SUMMARY

RNA-directed DNA polymerases from several xenotropic and ecotropic type-C murine viruses were compared. On sedimentation velocity gradients polymerases from Rauscher murine leukemia virus, AT-124 virus, New Zealand Black virus, and BALB: virus 2 appeared at identical positions as single peaks. Immunological comparisons using antisera against DNA polymerases from Rauscher virus and New Zealand Black virus failed to demonstrate significant differences among the viral enzymes tested.

INTRODUCTION

Recently, a previously unknown class of viruses was described (1). These viruses were termed "xenotropic" because of their apparent inability to infect cells of their species of origin, but ability to grow well in cells of other species (1). Xenotropic viruses appear to be "endogenous", i.e. their genetic information is within the DNA genome of each cell of the animal and under cellular control mechanisms.

Several strains of mice have yielded xenotropic viruses. Levy (1) reported evidence for xenotropic virus in New Zealand Black (NZB), NIH Swiss and C57/B16J mice by cocultivation with nonvirus-yielding rat cells transformed by Harvey sarcoma virus. Todaro et al. (2) obtained a possible NIH Swiss xenotropic virus, the AT-124, by passing human rhabdomyosarcoma cells in NIH Swiss mice immunosuppressed with antithymocyte serum. Aaronson and Dunn (3)

*Abbreviations used: R-MuLV, Rauscher murine leukemia virus; Ki-MuLV, Kirsten murine leukemia virus; FeLV, feline leukemia virus; NZB, New Zealand Black; IgG, Immunoglobulin G.

reported that BALB: virus 2, which grows in rat kidney cells but not in mouse cells, was spontaneously activated from K-BALB/3T3 cells.

In the present report we have compared the RNA-directed DNA polymerases of xenotropic and ecotropic viruses. All viral polymerases appeared to be of similar molecular weight, and we found little or no difference among the viral polymerases tested using specific antibody enzyme inhibition assays.

MATERIALS AND METHODS

Viruses and antisera. R-MuLV was obtained from Litton Bionetics, Inc. AT-124 virus, BALB: virus 2 (BC 177), and FeLV (Rickard) were purchased from Electro-Nucleonics. NZB virus was provided by Flow Laboratories. Antisera were prepared in a goat against R-MuLV DNA polymerase purified by oligo dT-cellulose column chromatography and were supplied by Roger Wilsnack, Huntington Laboratories. Preimmune serum from the same animal was also provided as control. We used standard chromatographic procedures on DEAE-cellulose to separate the immunoglobulin G (IgG) fraction from these sera. Guinea pig IgG prepared against purified NZB virus polymerase was obtained from Robert Toni, Flow Laboratories. A commercial guinea pig IgG preparation (Pentex) was used as control.

Glycerol gradients. 100 µl of virus lysate was layered onto 4 ml 10-30% glycerol gradients containing 500 mM KCl, 2 mM dithiothreitol, 10 mM Tris-HCl (pH 8), 2 mM magnesium acetate, and 0.5% Triton X-100. Gradients were centrifuged 15 hr. in a Beckman SW56Ti rotor at 50,000 rpm. The bottom of each tube was punctured and 20 14-drop fractions were collected from each gradient.

DNA polymerase and enzyme inhibition assays. Polymerase assays were done as previously described (4). The enzyme inhibition assay is described in legend of Table 1.

RESULTS

Glycerol sedimentation velocity gradient profiles of RNA-directed DNA polymerases from R-MuLV, NZB virus, AT-124, and BALB: virus 2 are shown in Fig. 1. All the viral polymerases appeared as single peaks which sedimented slightly faster than a bovine serum albumin marker, consistent with a molecular weight of approximately 70,000, as has been reported for R-MuLV (5). NZB virus DNA polymerase often appeared as two peaks on glycerol gradients. This occurred under what seem to be identical conditions to those giving us a single peak. The two enzyme forms were separated by only a fraction or two and were both slightly more rapidly sedimenting than the expected position. The other viral polymerases never exhibited this behavior, which may be due to aggregation perhaps because of a concentration effect or differences in preparation of this

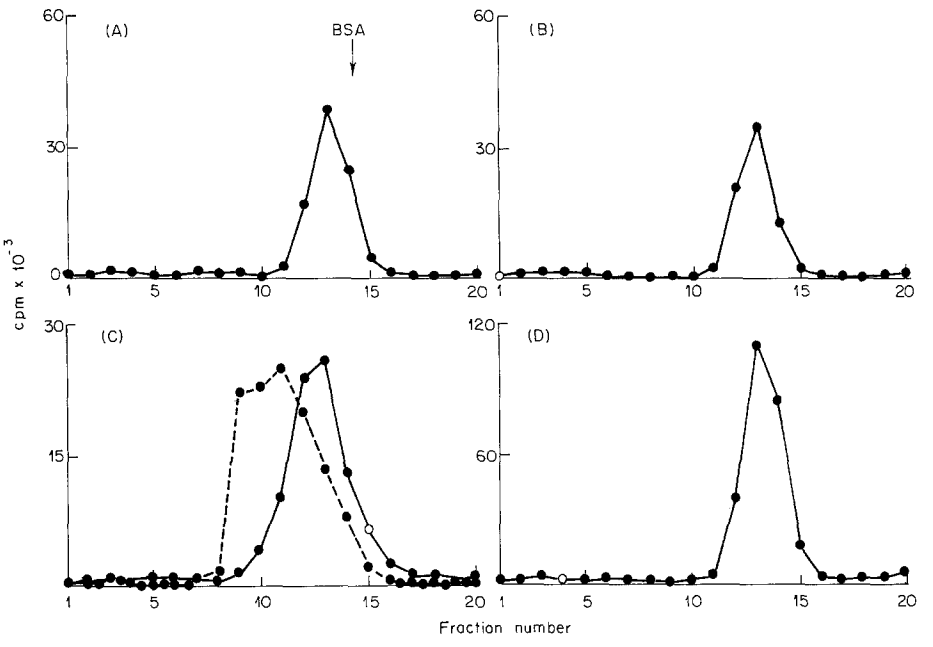

Figure 1. Sedimentation behavior of viral RNA-directed DNA polymerases on 10 → 30% glycerol gradients. The bottom of the gradient is fraction 1. A. R-MuLV. B. AT-124 virus. C. NZB virus. The dotted line indicates enzyme profile often obtained under apparently identical conditions. D. BALB: virus 2. The arrow in A indicates the sedimentation position of a bovine serum albumin marker.

virus. The multiple enzyme peaks were seen with both freshly prepared and frozen NZB virus.

Using the peak gradient fraction of each polymerase, enzyme inhibition assays were done with IgG from antisera made against purified DNA polymerases of NZB virus and R-MuLV. The results are shown in Table 1. Goat anti-R-MuLV polymerase IgG showed no cross reaction with the low molecular weight mouse cellular DNA polymerase which uses $(dA)_n \cdot (dT)_{10}$ as template-primer, nor was there much reaction with FeLV polymerase. Polymerase from RD-114, a feline endogenous virus, however, was essentially indistingushable from R-MuLV polymerase, which was inhibited 93% by the homologous antibody. DNA polymerases from NZB virus, BALB: virus 2, and AT-124 virus were inhibited 88, 94, and 90%, respectively. Guinea pig anti-NZB virus polymerase IgG inhibited NZB virus,

TABLE 1

DNA polymerase source[1]	% Inhibition	
	anti-R-MuLV polymerase IgG[2]	anti-NZB virus polymerase IgG[3]
R-MuLV	93	54
AT-124	90	41
NZB virus	88	48
BALB: virus 2	94	47
FeLV (Rickard)	26	14
RD-114	87	4
mouse uterus	1	6

Table 1. Effect of anti-R-MuLV polymerase IgG and anti-NZB virus polymerase IgG on DNA polymerases from various sources. Polymerases were adjusted to approximately equal activities and preincubated 30 min. on ice with indicated amounts of antisera in the presence of enzyme assay mixture minus TTP and template-primer. To start the reaction, TTP and $(rA)_n \cdot (dT)_{12-18}$ were added, and DNA polymerase activity remaining was determined at 37°. % inhibition was calculated by comparing activity at each antibody level with activity using control IgG at equal concentration.

[1]Viral polymerases were peak fractions from glycerol gradients. The mouse uterine DNA polymerase was the enzyme using $(dA)_n \cdot (dT)_{10}$ as template-primer and purified from AKR mouse uteri on Sephadex G-100 and hydroxylapatite.

[2]3.7 µg goat IgG.

[3]2.2 µg guinea pig IgG.

R-MuLV, AT-124 virus, and BALB: virus 2 polymerases 48, 54, 41, and 47%, respectively, but gave little inhibition of polymerases from mouse, FeLV, or RD-114. The two more rapidly sedimenting forms of NZB virus polymerase were indistinguishable from the single peak NZB polymerase using antisera against both NZB and R-MuLV polymerases.

DISCUSSION

Considerable interest has developed in xenotropic viruses. They have not been shown to cause any disease and may be involved in normal mammalian physiology. Our laboratory, using biochemical, immunological, and electron microscope techniques, has demonstrated a regular pattern of expression of type-C virus in the mouse uterus during gestation (manuscript in preparation). These viruses appear to be xenotropic by several criteria. After discovery of xenotropic viruses, it was obvious to ask in what way are they different from other type-C viruses. One can visualize surface membrane differences from previously known mouse leukemia viruses that could account for host range changes. Benveniste and Todaro (6), however, found only 48%, 25%, and 58% homology between AT-124 virus RNA and [^{3}H] DNA probes made from a BALB/c endogenous virus, R-MuLV, and Kirsten-MuLV (Ki-MuLV) by hybridization. In our study, no distinction was apparent among DNA polymerases of R-MuLV, AT-124 virus, and BALB: virus 2, in spite of the low degree of nucleic acid sequence homology noted above (6). Interestingly, although mouse xenotropic and ecotropic viral polymerases seem indistinguishable, the same is not true of feline viruses. As shown in Table 1, anti-R-MuLV polymerase IgG strongly inhibits the enzyme from RD-114 virus but not from FeLV. We reported earlier (7) that DNA polymerases from RD-114 and FeLV are strikingly different in inhibition assays using anti-RD-114 polymerase antisera. These differences are not apparent with anti-NZB virus polymerase antisera, however. Presumably, this preparation has antibodies directed to mouse-specific antigenic sites on the polymerases. Todaro et al. (2) reported similarities between polymerases from AT-124 and R-MuLV using anti-R-MuLV polymerase antiserum. Antiserum towards a xenotropic viral polymerase was not tested. In contrast to our results, the antisera of Todaro et al. (2) to R-MuLV polymerase more strongly inhibited FeLV polymerase than that of RD-114, though neither was strongly inhibited. It is conceivable that small differences exist between mouse virus polymerases that are not detected by our antisera.

In addition to the immunological similarities found, DNA polymerase activity from AT-124 virus, NZB virus, and BALB: virus 2 sedimented as a single peak and had the same sedimentation coefficient as the enzyme from R-MuLV, indicating similar molecular weights. DNA polymerase from NZB virus, however, often appeared as two peaks, both of which were more rapidly sedimenting than the other viral enzymes, suggesting larger molecular weights than polymerases from other viruses. These two enzymes could be artifacts. Wu et al. (8) have reported a possible dimer form of R-MuLV polymerase, although this form appears not to occur under the high salt conditions used in our experiments. A virally related DNA polymerase we have reported in mouse tissue (4) also is a higher molecular weight enzyme than that from purified virus.

Stephenson et al. (9) reported that change in host range characteristics from "mouse-tropic" to xenotropic occurred after prolonged growth of R-MuLV or Ki-MuLV in human cells. The altered properties were explained in terms of recombination of endogenous and exogenous viruses. Indeed, hybridization experiments suggested that the viruses with altered host range had lost sequences of the original exogenous virus and had acquired additional sequences identical or quite similar to those of known xenotropic viruses (NIH and BALB: virus 2). Our results are consistent with the hypothesis that xenotropic and ecotropic mouse viruses share a common precursor.

There are obviously a number of important questions to be answered concerning xenotropic viruses. In general, the evidence for the endogenous nature of mouse xenotropic viruses is indirect, e.g. immunological. Direct proof that the viral genome exists as a DNA provirus in every cell requires at least the demonstration of the requisite viral sequences in DNA isolated from tissues of the animal in question. Caution is required in extrapolating data from tissue culture systems to the intact animal, and special care must be used when assigning origins to viruses obtained from cells that have been in contact with foreign cells.

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