JOURNAL OF VIROLOGY, Nov. 1972, p. 959-964
Vol. 10, No. 5

Serological Analysis of the Deoxyribonucleic Acid Polymerase of Avian Oncornaviruses

II. Comparison of Avian Deoxyribonucleic Acid Polymerases

ROBERT C. NOWINSKI, K. F. WATSON, A. YANIV, AND S. SPIEGELMAN

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706, and the Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received for publication 11 July 1972

Monospecific antiserum prepared against the isolated deoxyribonucleic acid (DNA) polymerase of avian myeloblastosis virus (AMV) neutralized the endogenous ribonucleic acid-instructed DNA polymerase activity of detergent-disrupted virus. The viral polymerase was serologically unrelated to the seven major structural polypeptides of AMV. Furthermore, the viral enzyme was distinguished from normal cellular DNA polymerases by serological criteria; thus, antiserum against the viral enzyme neutralized its homologous antigen but not normal cellular DNA polymerases. Neutralization by antibody of viral DNA polymerase activity was observed with all avian leukemia-sarcoma viruses tested, irrespective of viral antigenic subtype. The DNA polymerase activity of avian reticuloendotheliosis virus, and of a variety of mammalian oncornaviruses, was not neutralized by antisera against the AMV polymerase. Immunological analysis of the RSVα(O) mutant, which is deficient in DNA polymerase activity, shows this mutant to lack demonstrable polymerase antigen. Viral polymerase was identified by immunofluorescence as a cytoplasmic constituent in virus-producing chicken cells; polymerase antigen was not detected in uninfected (gs^-) chicken cells.

Antibodies prepared against the deoxyribonucleic acid (DNA) polymerase of oncornaviruses are capable of neutralizing both ribonucleic acid (RNA)- and DNA-instructed polymerase activities (1, 6, 13, 16, 19, 22). The specificity of this reaction is analagous to that observed with the group-specific (gs) antigens (5, 12) of these viruses; thus, two classes of antigens are identified with the polymerase—those that are restricted to oncornaviruses of a single species, and those that are shared by oncornaviruses of more than one species (1, 19). Polymerases of all oncornaviruses possess antigens of the species-specific type, whereas only the polymerases of the leukemia-sarcoma viruses of mammalian origin have been shown to possess an antigen of the interspecies type. On the basis of immunological analysis of polymerases, several serologically distinct classes of oncornaviruses can be identified—the avian leukosis-sarcoma viruses, the lower mammalian leukemia-sarcoma viruses, the primate leukemia-sarcoma viruses, and the mouse mammary tumor viruses (1, 13, 19). Although the classification of viruses by either gs antigens or polymerase antigens is similar, these viral constituents have been shown to reside on different proteins. In most instances, however, where these viral constituents have been studied serologically, the analysis was performed with antibody prepared against partially purified enzymes or with polyvalent gs antisera.

In the preceding report (22), we described the preparation of a monospecific antiserum against purified DNA polymerase of avian myeloblastosis virus (AMV) and characterized the reaction of this antiserum with purified viral enzyme. In this report, we extend these studies to include a serological comparison of the AMV DNA polymerase to DNA polymerases from a variety of avian and mammalian oncornaviruses and from normal avian cells.

MATERIALS AND METHODS

Viruses. AMV was obtained from the blood of chicks in the terminal stage of myeloblastic leukemia and supplied by J. W. Beard, Duke University, Durham, N.C. Rous-associated virus-2 (RAV-2), a mixture of Bryan strain of Rous sarcoma virus and RAV-2 (BHT[RAV-2]), RSV B77 strain, the Schmidt-Rupin strain of RSV (RSV-SR), and the reticuloendotheliosis avian viruses (REV) were kindly pro-

vided by H. Temin, McArdle Laboratory, University of Wis., Madison, and were purified as previously described (3, 9). Purified RSVα(O) mutant, deficient in DNA polymerase activity, was kindly provided by H. Hanfusa, New York City Public Health Research Institute.

The Mason-Pfizer mammary tumor virus was obtained from supernatant fluids of NC-37 cells and partially purified by the Charles Pfizer Co., Maywood, N.J. Rauscher leukemia virus was obtained as a 10-fold mouse plasma concentrate, and feline leukemia virus (FeLV) was obtained from feline cells grown in suspension culture at the laboratory of R. Rickard, Cornell University, Ithaca, N.Y. Mouse mammary tumor virus was isolated from RIII mice. Visna virus was kindly supplied by Donald Harter, Department of Neurology, Columbia University College of Physicians and Surgeons, New York. Viruses were isolated as previously described (17, 18, 20).

Standard polymerase assay. All virus suspensions were disrupted with 0.2% Nonidet P-40 for 30 min at 0 C. Reaction mixtures (100 μliters) for homopolymer-instructed reactions contained the following in micromoles: tris(hydroxymethyl)aminomethane-hydrochloride (*p*H 8.2 at 25 C), 5.0; potassium chloride, 6.0; magnesium chloride, 0.6; dithiothreitol, 0.04; deoxyadenosine 5′-triphosphate, 0.02; radioactively labeled thymidine 5′-triphosphate (TTP), 0.01; synthetic polydeoxythymidylate-polyriboadenylate (poly dT·poly rA) template, 1.2×10^3 pmoles of polymer phosphate per strand; and detergent-disrupted viral DNA polymerase activity.

After incubation at 37 C for 60 min, the reaction mixtures were terminated at 0 C with the addition of cold 5% trichloroacetic acid. The acid-precipitable radioactivity was collected on nitrocellulose filters and counted in 0.4% 2,5-bis-2-(5-*tert*-butylbenzoxazolyl)thiophene in toluene. The specific activity of ^{3}H-TTP was 300 to 400 counts per min per pmole.

Neutralization assay for viral DNA polymerase. Purified viral suspensions were disrupted in 0.2% Nonidet P-40 detergent for 30 min at 0 C. To standardize the antigen content, each viral preparation was first tested for enzyme activity. One unit of virion DNA polymerase activity was defined as the amount of detergent-disrupted virus suspension necessary to render acid-precipitable 1 pmole of ^{3}H-TTP per min with poly dT·poly rA as template primer in the standard polymerase assay.

In the neutralization test, one unit of disrupted viral-enzyme activity was preincubated with immunoglobulin G (IgG) protein for 15 min at 37 C in a total volume of 60 μliters as previously described (22), and then tested in the standard DNA polymerase assay. The acid-precipitable radioactivity represents the the DNA polymerase activity not neutralized by antibody. Under the conditions described, the incorporation of ^{3}H-TTP was linear for more than 90 min.

DNA polymerase neutralization-inhibition assay. In a reaction mixture of 50 μliters containing 0.01 M Tris-hydrochloride (*p*H 8.2) and 0.15 M potassium chloride, 3 μg of anti-AMV DNA polymerase IgG or normal rat IgG were mixed with a given amount of test virus suspension that had been previously disrupted with 0.2% Nonidet P-40 for 30 min at 0 C. These are then incubated for 60 min at 37 C (incubation I), which was sufficient condition to enzymatically inactivate the test virus suspension, but not to antigenically denature the polymerase. Glycerol (final concentration, 20%) and bovine serum albumin (50 μg) were added to the reaction mixture, followed by the addition of one activity unit (*see* Materials and Methods) of detergent-disrupted AMV DNA polymerase activity, and the mixture (70 μliters) was then incubated at 37 C for 60 min (incubation II). To complete the assay, the volume of the mixture was increased to 110 μliters with the addition of standard polymerase assay components. Upon incubation at 37 C, 25-μliter samples were removed at 0, 30, 60, and 90 min for analysis of AMV DNA polymerase activity. Thus, the residual antibody activity remaining after incubation I was measured by its ability to neutralize AMV DNA polymerase activity in incubation II.

To evaluate the neutralization-inhibition assay, all tests included: (i) test virus suspension and normal rat IgG in incubation I without the addition of AMV enzyme in incubation II. This control showed that the DNA polymerase activity of the test virus suspension was deactivated during incubation I and was, therefore, not contributing activity in the final polymerase assay. (ii) Rat anti-AMV DNA polymerase IgG alone in incubation I with one unit of AMV enzyme activity added for incubation II. This control established the level of neutralization by the given amount of anti-AMV DNA polymerase IgG. (iii) Test virus suspension and normal rat IgG in incubation I with one unit of AMV enzyme activity added for incubation II. This control showed the effect of rat IgG and test virus suspension from incubation I on the AMV DNA polymerase activity added in incubation II and established the optimal value of activity to be reached by inhibition of neutralization. (iv) Test virus suspension and anti-AMV DNA polymerase IgG in incubation I with one unit of AMV enzyme activity added for incubation II. Test virus suspension that was antigenically related to AMV DNA polymerase absorbed the anti-AMV DNA polymerase IgG in incubation I, reducing the amount available for neutralization of AMV enzyme in incubation II.

Immunofluorescence test. The indirect immunofluorescence test on fixed cells was used for the localization of intracellular viral antigens (8). Monolayers of chicken embryo fibroblasts were grown for 16 hr in individual wells on glass microscope slides and fixed by immersion of the slides in acetone at room temperature for 10 min. Fluorescentated goat anti-rat gamma globulin (Hyland Laboratories) was used at 1:30 dilution; rat anti-AMV DNA polymerase serum was used at a 1:20 dilution.

Preparation of antisera against the structural proteins of RSV-SR virus. RSV-SR virus was purified and viral structural proteins were isolated as described previously (4, 11). For the preparation of antisera, each isolated viral protein component was subjected to a second cycle of gel filtration chromatography prior to immunization. Procedures for immunization and fractionation of antisera have been described (11).

RESULTS

Serological comparison of DNA polymerases of avian and mammalian oncornaviruses. Immunoglobulins prepared against the purified DNA polymerase of AMV neutralized the DNA polymerase of AMV and all avian leukemia and sarcoma viruses tested, irrespective of viral membrane antigen subtypes. Figure 1 shows neutralization data with avian leukemia (AvLV) and sarcoma viruses of the A(AMV), B(RAV-2), C(B77), and D(RSV-SR) subgroups.

Similar neutralization assays with the virus REV, a DNA polymerase-containing avian virus (2, 14, 21), were consistantly negative (Fig. 1). Thus, the independent origin of REV from AvLV is supported by the findings that REV does not contain AvLV gs antigens (2, 14, 21) and that the DNA polymerase of REV is serologically unrelated to the DNA polymerase of AvLV.

Antibody prepared against the isolated polymerase of AMV was also tested for neutralization activity against the DNA polymerase of a variety of mammalian oncornaviruses; Figure 1 shows that this antibody did not affect the DNA polymerases of murine leukemia virus, murine mammary tumor virus, feline leukemia virus, visna virus, or the Mason-Pfizer monkey virus. Thus, this antiserum shows group specificity for the DNA polymerase of avian oncornaviruses but does not react with the DNA polymerase of the mammalian oncornaviruses.

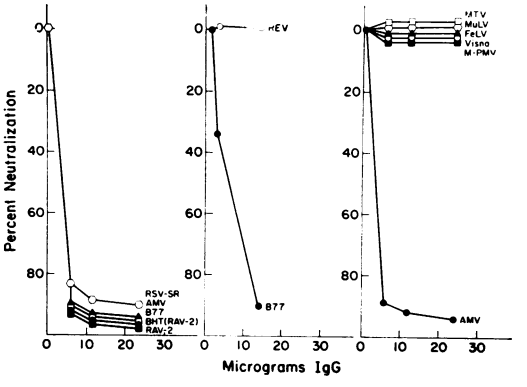

FIG. 1. *Neutralization of viral DNA polymerase with IgG from rat anti-AMV DNA polymerase serum. Left, Neutralizing effect of increasing amounts of rat anti-AMV DNA polymerase IgG on one activity unit (see Materials and Methods) of disrupted RSV-SR (hexagonal sign), AMV (▲——▲), B77 (□——□), BHT(RAV-2) (●——●), and RAV-2 (■——■) virion DNA polymerase. Center, Neutralization of REV (○——○) and B77 (●——●) DNA polymerase by anti-AMV DNA polymerase IgG. Right, Neutralizing effect of the same IgG fraction on one activity unit of disrupted mouse mammary tumor virus □——□, murine leukemia virus (hexagonal sign), FeLV (▲——▲), visna (○——○), Mason-Pfizer mammary tumor virus (■——■), and AMV (●——●) virion DNA polymerase. Control rat serum IgG was tested at comparable concentrations for determination of percent neutralization. The neutralization assay presented in the center figure was kindly provided by C. Y. Kang and H. M. Temin (10).*

Serological comparison of the avian viral DNA polymerase to viral structural proteins. Antisera prepared in rats against proteins of RSV-SR that were isolated by gel filtration in guanidine hydrochloride (*see* Fig. 4 in reference 22 for a representative separation) were tested for neutralization activity against purified DNA polymerase of AMV. None of these antisera showed inhibitory activity for the enzyme; these same antisera, however, had precipitating antibody against their respective antigens (Table 1).

A similar serological analysis (*see below*) showed that the viral DNA polymerase was antigenically unrelated to DNA polymerases of normal chicken cells.

Serological comparison of viral DNA polymerases by a neutralization-inhibition assay. To develop a technique for the quantitative comparison of dissimilar virus preparations, the enzyme neutralization assay was adapted to include a quantitative antibody-absorption step. In this assay, a constant amount of immunoglobulin (shown by titration to be the minimal amount of antibody required to neutralize 60 to 70% of the polymerase activity in a standard neutralization assay) was first preincubated with a given amount of test

TABLE 1. *Enzyme neutralization assay with antisera prepared against the structural proteins of RSV-SR*[a]

Antiserum	IgG (μg)	Residual enzyme activity (%)
Normal rat serum	42.0	100[b]
Rat anti-AMV DNA polymerase	42.0	2.2
Rat anti-RSV-SR m1	43.5	102
Rat anti-RSV-SR m2	41.4	98
Rat anti-RSV-SR gs1	45.4	102
Rat anti-RSV-SR gs2	43.5	113
Rat anti-RSV-SR gs3	42.2	116
Rat anti-RSV-SR gs4	46.5	138
Rat anti-RSV-SR p5	45.6	126

[a] For details of viral proteins see references 4 and 12.

[b] Enzyme activity (from 0.2 μg of disrupted AMV) is normalized to 100 for the sample incubated with IgG from normal rat serum.

(detergent-disrupted) virus suspension, and then tested for residual antibody activity in a standard neutralization assay. This method, referred to as the neutralization-inhibition assay, is illustrated in Fig. 2. The upper left figure shows a kinetic analysis of a neutralization-inhibition assay with AMV as the test virus suspension. The upper right figure summarizes the results of increasing the amount of the AMV suspension incubated with a constant amount of anti-AMV DNA polymerase IgG. Neutralization of AMV DNA polymerase activity was totally inhibited by preincubation of antibody with 1 μg of virion protein. The increased sensitivity gained by absorbing antibody close to its end point of activity permits the detection of viral polymerase antigen with less than 0.5 μg of total viral protein.

For a serological comparison, other avian and mammalian oncornaviruses were tested for the ability of their respective DNA polymerase to absorb anti-AMV DNA polymerase antibody. Figure 2 describes typical results for RAV-2 and a mammalian virus (FeLV). It can be concluded that the avian oncornaviruses contain immunologically related DNA polymerase antigen, whereas the mammalian oncornaviruses exhibit no antigenic similarity. This specificity agrees with the results of the neutralization studies.

Analysis of the RSVα(O) mutant deficient in DNA polymerase activity. The RSVα(O) mutant is a noninfectious, nontransforming virus that is deficient in DNA polymerase activity (7, 15). We have tested an isolate of this mutant (kindly provided by H. Hanafusa) in parallel with AMV for the presence of immunologically detectable enzyme. Figure 3 shows that, within the limits of the neutralization-inhibition test, the RSVα(O) mutant is at least 60-fold deficient in respect to polymerase antigen; preincubation of anti-AMV DNA polymerase IgG with 0.5 μg of AMV protein significantly inhibited the antibody activity, whereas 30 μg of RSVα(O) protein had no effect. Hence, the failure to find DNA polymerase in this mutant is due to the physical absence of enzyme in the virion, rather than the production of an enzymatically inactive protein.

Detection of viral DNA polymerase in cells by immunofluorescence. Rat anti-AMV DNA polymerase serum was tested by immunofluorescence on chicken cells that were transformed and producing avian sarcoma virus (B77). The fluorescence reaction was restricted to the cytoplasm (Fig. 4). Generally, the fluorescence was punctate, and, although it was a relatively weak reaction, it was similar in appearance to that observed with other viral antigens (i.e., the gs antigens [8, 11]). Absorption tests with antiserum diluted near the end point of fluorescence showed that the anti-

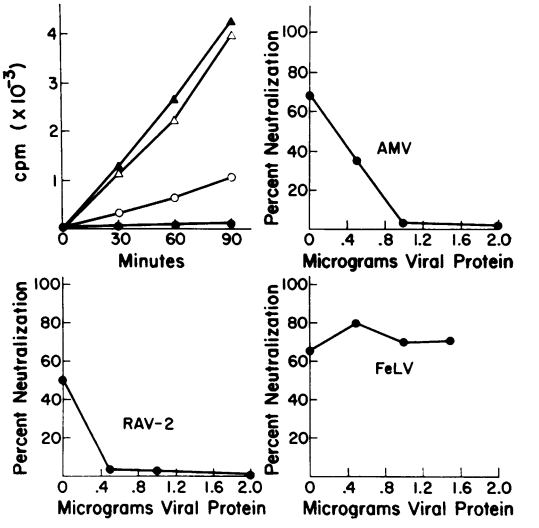

FIG. 2. *Neutralization-inhibition assay with IgG from rat-anti AMV DNA polymerase serum. Upper left, Kinetic analysis of a neutralization-inhibition assay (see Materials and Methods) with detergent-disrupted AMV virions (1 μg) as the test virus suspension. Closed circles indicate that the detergent-disrupted AMV protein added in incubation I for absorption of antibody does not contribute any enzyme activity to the one activity unit of AMV enzyme added in incubation II. Open circles show that the anti-AMV DNA polymerase IgG added alone in incubation I neutralizes 70% of the AMV enzyme activity added in incubation II. Closed triangles show the control reaction where normal rat IgG and test virus suspension in incubation I are seen to have a negligible effect on the enzyme activity in incubation II. Open triangles illustrate the absorption of anti-AMV DNA polymerase IgG in incubation I by 1 μg of detergent-disrupted AMV protein. Upper right, Results obtained with increasing amounts of detergent-disrupted AMV protein in incubation I. Lower left, Ability of increasing amounts of detergent-disrupted RAV-2 virion protein in incubation I to inhibit the neutralizing ability of anti-AMV DNA polymerase IgG in incubation II. Lower right, Inability of FeLV virion protein to inhibit the enzyme neutralization.*

AMV DNA polymerase activity could be effectively absorbed by purified viral enzyme but not by other viral proteins.

Rat anti-AMV DNA polymerase serum did not react with normal uninfected gs⁻ chicken cells in immunofluorescence tests.

DISCUSSION

Immunization of rats with the highly purified DNA polymerase of AMV (9) resulted in the formation of monospecific antisera (22). The isolated enzyme used for immunization showed DNA polymerase activity directed by RNA, DNA, and RNA-DNA hybrid templates, and,

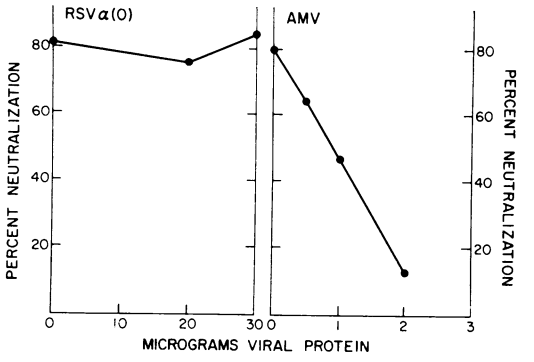

FIG. 3. *Neutralization-inhibition assay with RSVα(0). IgG (4 μg) from rat anti-AMV DNA polymerase serum was preincubated with varying amounts of detergent-disrupted RSVα(0) or AMV, and then tested for residual neutralization activity on AMV DNA polymerase. Preincubation of IgG with 0.5 μg of AMV protein significantly inhibited the antibody activity, whereas 30 μg of RSVα(0) protein had no effect.*

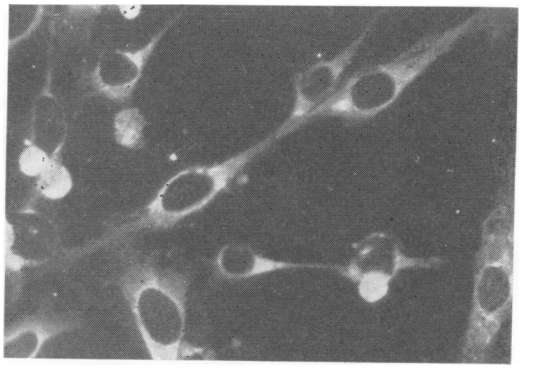

FIG. 4. *Immunofluorescent detection of viral DNA polymerase in RSV-infected cells. Chicken embryo cells infected with B77 virus were tested by indirect immunofluorescence for viral DNA polymerase. Antigen was detected only in the cytoplasm. For the purpose of photography, the intensity of this reaction was increased over that observed by eye by controlling the time of exposure. This same antiserum did not react with normal chicken embryo cells.*

similarly, antisera prepared against this enzyme inhibited each type of enzymatic activity (22). Antigens found in AMV polymerase also are common to other avian leukemia-sarcoma viruses, but are not present in REV. As described by others (1, 13), the common antigenicity of avian viral polymerases does not extend to the DNA polymerases of mammalian oncornaviruses.

Distinctions between the viral DNA polymerase and normal chicken cell DNA polymerases can be demonstrated immunologically. A serological analysis of the viral DNA polymerase with respect to normal cellular DNA polymerase was kindly provided by S. Mizutani, C. Y. Kang, and H. Temin. In their studies, our rat anti-AMV DNA polymerase serum did not inhibit a sedimentable endogenous RNA-instructed DNA polymerase activity isolated from chicken embryos or fibroblasts (10). Similarly, this antiserum did not have inhibitory activity for a soluble, cellular DNA polymerase (characterized by a sedimentation coefficient of 10S) isolated from normal chicken embryos or fibroblasts. In preliminary studies (by S. Mizutani and R. Nowinski), it has been shown that an antiserum prepared in the rat against this soluble cellular DNA polymerase neutralizes its homologous antigen activity but has no effect upon the viral DNA polymerase activity. The serological distinctions between viral and normal cellular DNA polymerases was demonstrated also by immunofluorescence. Thus, antisera prepared against the viral DNA polymerase reacted with virus-infected cells, but not with noninfected cells. In a similar study, the DNA polymerase of murine leukemia virus had been found to be serologically unrelated to normal murine cellular DNA polymerase (16).

Antisera prepared against isolated avian sarcoma virus proteins were found to have no neutralization activity against purified DNA polymerase of AMV. This finding was somewhat surprising, since under the conditions of chromatography in 6 M guanidine hydrochloride and reducing agents it would be expected that the DNA polymerase would dissociate into its subunits, and that these would co-chromatograph (on the basis of molecular weight) with viral proteins m1 and m2 (9, 12). The failure to find antipolymerase activity with these antisera may be a result of inadequate antigen concentration in the fractions used for immunization, or perhaps that the antigenicity of the enzyme is dependent upon an intact enzyme complex.

A method, referred to as the neutralization-inhibition assay, was developed to include a quantitative antibody-absorption step. With this technique, it was possible to detect less than 2.5 ng of viral polymerase in a disrupted virion suspension. Serological analysis of the RSVα(O) mutant, an avian sarcoma virus deficient in DNA polymerase activity (7, 15), showed the actual absence of enzyme in this virus. Further substantiation of this point has been described by Hanafusa et al. (7a). Because the RSVα(O) isolate of Robinson and Robinson (15) does have some DNA polymerase activity, it is possible that their isolate is an example of a specific defect in the polymerase enzyme. Further studies should clarify this point.

Perhaps the most important aspect of this

study is the development of an antiserum that is monospecific for the viral DNA polymerase. With this antiserum, a reagent is now available that allows the direct study of intracellular viral polymerase, without the interference of other cellular DNA polymerase activities. A first step in this analysis was the demonstration by immunofluorescence that viral polymerase resides in discrete cytoplasmic regions. This approach can be extended to include the detection of viral polymerase in other cell types, and to the analysis of the kinetics of viral enzyme synthesis during the infectious cycle.

ACKNOWLEDGMENT

This work was supported by Public Health Service Special Virus Cancer Program Contract 70-2049, research grant CA-02 232, and Postdoctoral Fellowship CA-38924, all from the National Cancer Institute.

LITERATURE CITED

1. Aaronson, S. A., W. P. Parks, E. M. Scolnick, and G. J. Todaro. 1971. Antibody to the RNA-dependent DNA polymerase of mammalian C-type RNA tumor viruses. Proc. Nat. Acad. Sci. U.S.A. 68:920–924.
2. Bose, H. R., and A. S. Levine. 1967. Replication of the reticuloendotheliosis virus (strain T) in chicken embryo cell culture. J. Virol. 1:1117–1121.
3. Carnegie, J. W., A. O. C. Deeney, K. C. Olson, and G. S. Beaudreau. 1969. An RNA fraction from myeloblastosis virus having properties similar to transfer RNA. Biochim. Biophys. Acta 190:274–284.
4. Fleissner, E. 1971. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. I. Avian leukemia-sarcoma viruses. J. Virol. 8:778–785.
5. Geering, G., T. Aoki, and L. J. Old. 1970. Shared viral antigens of mammalian leukemia viruses. Nature (London) 226:265–266.
6. Gerwin, B. I., G. J. Todaro, V. Zeve, E. M. Scolnick, and S. A. Aaronson. 1970. Separation of RNA-dependent DNA polymerase activity from the murine leukemia virion. Nature (London) 228:435–438.
7. Hanafusa, H., and T. Hanafusa. 1971. Noninfectious RSV deficient in DNA polymerase. Virology 43:313.
7a. Hanafusa, H., D. Baltimore, D. Smoler, K. F. Watson, A. Yaniv, and S. Spiegelman. 1972. Absence of polymerase protein in virions of alpha-type Rous sarcoma virus. Science 177:1188–1191.
8. Hilgers, J., R. C. Nowinski, G. Geering, and W. Hardy. 1972 Detection of avian and mammalian oncogenic RNA viruses (oncornaviruses) by immunofluorescence. Cancer Res. 32:98–106.
9. Kacian, D. L., K. F. Watson, A. Burny, and S. Spiegelman. 1971. Purification of the DNA polymerase of avian myeloblastosis virus. Biochim. Biophys. Acta 246:365–383.
10. Kang, C. Y., and H. Temin. 1972. An endogenous RNA-directed DNA polymerase system in normal uninfected chicken embryos. Proc. Nat. Acad. Sci. U.S.A. 69:1550–1554.
11. Nowinski, R. C., E. Fleissner, N. H. Sarkar, and T. Aoki. 1972. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. II. Mammalian leukemia-sarcoma viruses. J. Virol. 9:359–366.
12. Nowinski, R. C., L. J. Old, P. O'Donnell, and F. K. Sanders. 1971. Serological identification of hamster oncornaviruses. Nature (London) 230:282–284.
13. Parks, W. P., E. M. Scolnick, J. Ross, G. J. Todaro, and S. A. Aaronson. 1971. Immunological relationships of reverse transcriptases from ribonucleic acid tumor viruses. J. Virol. 9:110–116.
14. Peterson, D. A., K. L. Bacter-Gabbard, and A. S. Levine. 1972. Avian reticuloendotheliosis virus (strain T). V. DNA polymerase. Virology 47:251–254.
15. Robinson, W. S., and H. L. Robinson. 1971. DNA polymerase in defective Rous sarcoma virus. Virology 44:457–462.
16. Ross, J., E. M. Scolnick, G. J. Todaro, and S. A. Aaronson. 1971. Separation of murine cellular and murine leukemia virus DNA polymerases. Nature (London) 231:163–167.
17. Schlom, J., and S. Spiegelman. 1971. DNA polymerase activities of virions isolated from a spontaneous mammary carcinoma from a Rhesus monkey. Proc. Nat. Acad. Sci. U.S.A. 68: 1613–1617.
18. Schlom, J., S. Spiegelman, and D. Moore. 1971. RNA-dependent DNA polymerase activity in virus-like particles isolated from human milk. Nature (London) 231:97–100.
19. Scolnick, E. M., W. P. Parks, G. J. Todaro, and S. A. Aaronson. 1972. Immunological characterization of primate C-type virus reverse transcriptases. Nature (London) 235: 35–40.
20. Spiegelman, S., A. Burny, M. R. Das, J. Keydar, J. Schlom, M. Travnicek, and K. Watson. 1970. Characterization of the products of RNA-directed DNA polymerase in oncogenic RNA viruses. Nature (London) 227:563–567.
21. Theilen, G. H., R. F. Zeigel, and M. F. Twiehaus. 1966. Biological studies with RE virus (strain T) that induces reticuloendotheliosis in turkeys, chickens, and Japanese quail. J. Nat. Cancer Inst. 37:731–743.
22. Watson, K. F., R. C. Nowinski, A. Yaniv, and S. Spiegelman. 1972. Serological analysis of the DNA polymerase of avian oncornaviruses. I. Preparation and characterization of monospecific antiserum with purified deoxyribonucleic acid polymerase. J. Virol. 10:951–958.