

Antigenic relatedness of the DNA polymerase of human breast cancer particles to the enzyme of the Mason-Pfizer monkey virus

(reverse transcriptase/human malignancy/oncornavirus/immunologic crossreactivity)

T. OHNO AND S. SPIEGELMAN

Institute of Cancer Research, College of Physicians and Surgeons of Columbia University, 701 West 168th Street, New York, New York 10032

Contributed by S. Spiegelman, March 7, 1977

ABSTRACT We have previously reported [Ohno, T., Sweet, R. W., Hu, R., DeJak, D. & Spiegelman, S. (1977) *Proc. Natl. Acad. Sci. USA* 74, 764-768] on the purification and characterization of the DNA polymerase from human breast cancer particles. Its preference for certain synthetic templates and its ability to use a viral RNA to fashion a faithful DNA transcript identify it as a reverse transcriptase similar to that found in the mouse mammary tumor virus and in the Mason-Pfizer monkey virus (MPMV). We report here that the human breast cancer enzyme crossreacts immunologically with the reverse transcriptase of MPMV. The crossreactivity was shown both by inhibition of enzyme activity and by complex formation between purified enzyme and isolated IgG against MPMV polymerase. No such interactions were observed with other oncornavirus reverse transcriptases of avian, murine, feline, or simian origin. Further, the IgG failed to neutralize the reverse transcriptases from human mesenchymal neoplasias (leukemias and lymphomas) or the activities of normal cellular DNA polymerases (α , β , γ).

Previous studies (1-7) have identified human breast tumor particles that exhibit many of the features characteristic of RNA tumor viruses. In addition to the expected size (600 S) and density (1.16 g/ml), these particles have an outer membrane and an inner one surrounding a "core" containing a DNA polymerase (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) (reverse transcriptase) complexed to a large (70 S) RNA exhibiting detectable homology to the RNAs of the mouse mammary tumor virus and of the Mason-Pfizer monkey virus (MPMV). The DNA polymerase of human breast cancer particles has been purified (8) as a 70,000-dalton protein with the following three features that together serve to distinguish viral reverse transcriptases from the known animal cellular DNA polymerases: (i) a strong preference for oligo(dT)-poly(rA) over oligo(dT)-poly(dA) as a template for the synthesis of poly(dT); (ii) the acceptance of the specific oligo(dG)-poly(rCm) as a template for the synthesis of poly(dG); and (iii) the ability to use a viral RNA (avian myeloblastosis virus) as a template to fashion a faithful DNA complementary copy.

The present investigation focuses on the antigenic properties of the human breast cancer reverse transcriptase with particular emphasis on possible crossreactivities with animal viral reverse transcriptases. This type of immunologic information has been used by tumor virologists to classify the oncogenic RNA viruses into the following distinct groups: (i) the avian leukemia-sarcoma viruses (9-11); (ii) the subprimate leukemia-sarcoma viruses (9-14); (iii) the nonhuman primate leukemia-sarcoma viruses (11, 13, 14); (iv) the mouse mammary tumor virus (9, 10, 12); and (v) a unique group composed of the MPMV and its close relatives (15).

In addition to its biologic and evolutionary implications, the identification of antigenic relationships among oncogenic agents from different species can generate useful immunologic re-

agents for the detection of viruses or their protein components. In particular, a demonstration of an antigenic relationship between a protein of the virus-like particles found in human neoplasias and a corresponding protein of a known animal virus could bypass the logistic difficulties of obtaining purified human particle proteins in the amounts required to produce the relevant antisera. This in turn could permit the development of a potentially useful diagnostic tool. Thus far, attempts along these lines have been confined to the leukemias. The reverse transcriptase found in leukemic cells has in some instances (16, 17) been neutralized by antibodies prepared against the reverse transcriptases of woolly monkey sarcoma virus and the gibbon ape leukemia virus.

We report here that the reverse transcriptase purified from human breast cancer particles crossreacts with antibodies produced by immunizing rabbits with purified reverse transcriptase of the MPMV isolated from a spontaneous mammary tumor of a rhesus monkey (4).

MATERIALS AND METHODS

Viruses. MPMV was propagated in a suspension culture of the normal human lymphocytic cell line NC-37 and concentrated at the J. L. Smith Memorial for Cancer Research (Pfizer, Inc.) as previously described (18). The virus was further purified by centrifugation through an 8-ml column of 20% (vol/vol) glycerol in 0.01 M Tris-HCl, pH 8.3/0.15 M NaCl/0.002 M EDTA onto a pad of 100% glycerol at $98,000 \times g$ for 60 min at 4° . The viral pellet was taken up in the same buffer and spun to equilibrium in a continuous 20-50% sucrose gradient in the buffer at $98,000 \times g$ for 16 hr. The particles banding between densities of 1.14 and 1.19 g/ml were collected, diluted, and centrifuged at $98,000 \times g$ for 45 min at 4° . The pellet was used immediately for DNA polymerase purification.

Avian myeloblastosis virus (BAI strain-A) was obtained from J. W. Beard; simian sarcoma virus (strain 1) from D. G. Deinhardt (Pfizer, Inc.); Friend leukemia virus and feline leukemia virus from D. Bolognesi; and Rauscher leukemia virus from E. H. Bernstein (University Labs). All viral concentrates were purified as described above.

Preparation of RNA-Instructed DNA Polymerase from Human Malignant Breast Tumor. Reverse transcriptase from human malignant breast tumor was prepared as previously described (8) from particles purified by isopycnic separation. After disruption by incubation in 0.2% (vol/vol) Triton X-100 for 15 min at 0° , some of the samples were analyzed for endogenous polymerase activity as well as oligo(dG)-poly(rC)- and oligo(dT)-poly(rA)-directed synthesis of poly(dG) and poly(rA), respectively. The disrupted virus density regions were chromatographed on a polyacrylamide agarose column (Ultrogel AcA44, LKB Co.), and the eluted enzyme peak was loaded on a phosphocellulose (Whatman p11) column. The enzyme was eluted with a 0.01-0.5 M potassium phosphate gradient and concentrated as described (8).

Abbreviation: MPMV, Mason-Pfizer monkey virus.

Preparation of Viral Regions from Human Leukemia and Hodgkin's Lymphoma Spleen. Splens from patients with human chronic lymphocytic leukemia, chronic myelogenous leukemia, or Hodgkin's lymphoma were used as the sources of viral density region preparations, and the polymerase activities were analyzed by endogenous kinetics as described (19).

Preparation of MPMV Polymerase. The MPMV pellet prepared as described above was resuspended in 0.05 M Tris-HCl, pH 9.2/0.001 M EDTA/2 M KCl, sonicated, and then centrifuged at $98,000 \times g$ for 120 min. The pellet was used to prepare purified DNA polymerase by column chromatography as described previously (19).

Preparation of Antiserum. Antiserum against MPMV DNA was induced in New Zealand White rabbits. Three cycles of immunization were required to achieve the desired titer of anti-polymerase IgG. In each cycle the enzyme (1×10^3 pmol of TMP incorporated per min) was emulsified with an equal volume of Freund's adjuvant and injected into the two hind footpads. This was followed by two additional similar inoculations given at 2-week intervals in the same sites.

Serum samples were fractionated by chromatography on a Sephadex G-200 with 0.1 M Tris-HCl, pH 8.0. Rabbit gamma globulins were identified serologically by immunodiffusion with goat antiserum to rabbit IgG. The relevant fractions were concentrated by ammonium sulfate precipitation (50% saturation) and dialyzed against 0.1 M Tris-HCl, pH 8.0. The protein concentration of the IgG fraction was measured by the Lowry procedure (20).

DNA Polymerase Assays. Assay mixtures for polymerase activity with synthetic polymer templates contained (in 100 μ l) 5 μ mol of Tris-HCl, pH 8.0; 0.5 μ mol of $MgCl_2$; 0.1 μ mol of dithiothreitol; and the following combinations of polymer and dNTPs: 0.4 μ g of oligo(dG)-poly(rC) or oligo(dG)-poly(rCm), 0.02 μ mol of dCTP and 1.0 nmol of [3H]dGTP (4000 cpm/pmol), 0.4 μ g of oligo(dT)-poly(rA) or oligo(dT)-poly(dA), 0.02 μ mol of dATP and 1.0 nmol of [3H]dTTP (4000 cpm/pmol). In reactions with oligo(dG)-poly(rCm), $MnCl_2$ (0.02 μ mol) replaced $MgCl_2$.

Assays using endogenous RNA contained (in 100 μ l) 5 μ mol of Tris-HCl, pH 8.0; 0.8 μ mol of $MgCl_2$; 0.1 μ mol of dithiothreitol; 10 μ g of actinomycin D (Sigma); 5 μ g of distamycin A (Calbiochem); 0.1 μ g of oligo(dT)₁₂₋₁₈; 0.1 μ mol each of dATP, dGTP, and dTTP; and 5 nmol of [3H]dCTP (1.5×10^4 cpm/pmol).

All reactions were incubated at 36° for 15–30 min as indicated and were terminated by the addition of 0.5 ml of cold 0.067 M sodium pyrophosphate/1 M sodium phosphate, pH 7.2, followed by 0.5 ml of cold 80% (vol/vol) trichloroacetic acid. Acid-insoluble radioactivity was collected on membrane filters and measured in a scintillation counter.

Terminal deoxynucleotide transferase activity was measured by the polymerization of [3H]dGTP in the absence of a complementary polymer template, the latter being replaced by 0.4 μ g of oligo(dG)₁₀₋₁₈.

All synthetic oligo- and polynucleotides were obtained from Collaborative Research, Inc.; tritiated dGTP, dTTP, and dCTP were obtained from New England Nuclear.

The Effect of Antibody on DNA Polymerase Activities. Reaction mixtures for the neutralization of DNA polymerase activity (total volume, 55 μ l) contained, in addition to 25 μ g of bovine serum albumin and DNA polymerase, the indicated amount (25–150 μ g) of purified IgG fraction. The buffer was 0.01 M Tris-HCl, pH 8.0/0.15 M KCl. After a 15-min incubation at 4°, a polymerase assay was carried out with oligo(dG)-poly(rC) as described above. In certain instances, the effect of antibody on the activity of the endogenous RNA was examined.

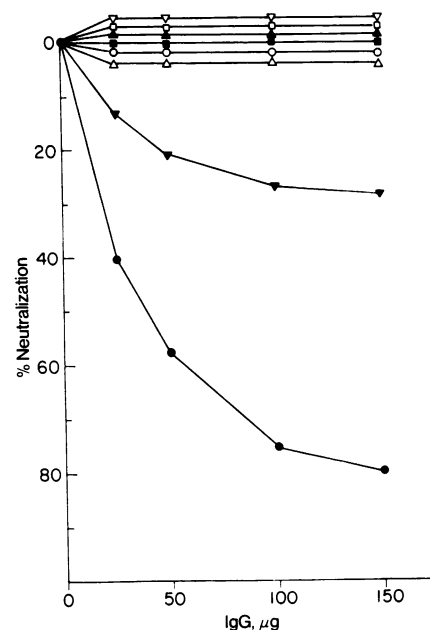


FIG. 1. Effect of anti-MPMV polymerase IgG on the DNA polymerase activities of animal oncornaviruses and of the human breast cancer particle. Standard polymerase reactions were performed as described in *Materials and Methods*, with oligo(dG)-poly(rC) as template. Reaction mixtures containing 25 μ g of bovine serum albumin and the indicated amounts of IgG were incubated at 36° for 15 min and then assayed for acid-precipitable radioactivity. Controls containing comparable amounts of normal rabbit serum IgG were run to determine the percentage neutralization. The DNA polymerase activities tested were derived from the following viruses or particles: avian myeloblastosis (∇), Rauscher leukemia (\blacktriangle), Friend leukemia (\blacksquare), feline leukemia (\square), mouse mammary tumor (\circ), woolly monkey sarcoma (\triangle), MPMV (\bullet), and human malignant breast tumor particle (\blacktriangledown). For display, the six activities not affected by the IgG are displaced slightly from their actual position; all were, within experimental error, at 100% of control activity.

Detection of Antibody-Enzyme Complexes in Glycerol Gradients. The concentrated enzyme (reverse transcriptase from MPMV or from human malignant breast tumors) fractions were diluted with 2 volumes of 0.1 M potassium phosphate, pH 8.0, containing bovine serum albumin (0.5 mg/ml), and the indicated amounts of purified IgG fractions were added. After incubation for 15 min at 4°, the samples were layered over a 10–30% glycerol gradient adjusted to 0.1 M potassium phosphate (pH 8.0), 0.002 M dithiothreitol, and 0.02% Triton X-100. The samples were sedimented at 48,000 rpm for 12 hr in a Spinco SW50-1 rotor at 1°. Fractions were collected dropwise from the bottom of the tubes and the enzyme activity was assayed as described above.

RESULTS

Comparison of the Effects of Anti-MPMV DNA Polymerase IgG on Various Polymerases. In these experiments, isopycnicly banded particles, purified as described in *Materials and Methods*, were used as a source of the DNA polymerase, and oligo(dG)-poly(rC) was used as the template. It is evident from Fig. 1 that the antibody inhibited the MPMV DNA polymerase more than 80% and achieved a 26% inhibition of the DNA polymerase associated with the human breast cancer particles. In contrast, no detectable effect was observed on the DNA polymerases of any of the other animal oncornaviruses, including avian myeloblastosis virus, Rauscher and Friend murine leukemia viruses, feline leukemia virus, a simian sarcoma virus, and murine mammary tumor virus.

Specificity of the Inhibition by the Anti-MPMV Poly-

Table 1. Effect of anti-MPMV polymerase IgG on endogenous reverse transcriptase activities of particles from human malignant tissues

Cells or tissues	Normal IgG	With BSA		With anti-MPMV IgG		With RNase	
	cpm	cpm	%	cpm	%	cpm	%
Breast cancer (exp. 1)	1862	1748	93.8	765	41.1	331	17.7
Breast cancer (exp. 2)	3682	3429	93.1	138	3.7	195	5.3
Breast cancer (exp. 3)	2340	2118	90.5	283	12.1	321	13.7
CML, spleen	3086	3110	100.7	2806	90.9	168	5.4
CLL, spleen	1540	1640	106.5	1621	105.2	126	8.2
Hodgkin's spleen	1261	1205	95.5	1018	80.7	186	14.8

Reaction mixtures (50 μ l) contained 50 μ g of anti-MPMV polymerase IgG, normal rabbit IgG, or bovine serum albumin (BSA), and detergent-disrupted particles. Incubation to permit complex formation with antibody was carried out for 15 min at 0°. The endogenous enzyme activity was then measured at 37° for 15 min as described in *Materials and Methods*. The sensitivity to RNase (80 μ g/ml) was also examined. CML = chronic myelogenous leukemia; CLL = chronic lymphocytic leukemia.

merase IgG. The next issue examined centered on whether the inhibition observed with the breast cancer particle enzyme was confined to this malignancy. As we have shown previously (19), spleens from patients with mesenchymal cancers constitute a convenient source of particle enzyme, and these were chosen for immunologic comparison. The particle fractions were prepared and the endogenous polymerase activities were assayed as previously described for human breast tumors (6, 8) and for spleens involved in mesenchymal neoplasias (19). At least five specimens of each kind of neoplastic tissue were examined, and typical results are shown in Table 1. Significant inhibitions are not seen with the particle enzymes derived from the leukemia and the lymphoma spleens. However, the breast cancer particulate enzymes were inhibited from 59% to more than 95%. Note that these endogenous reactions were more severely affected by the anti-MPMV polymerase IgG than the

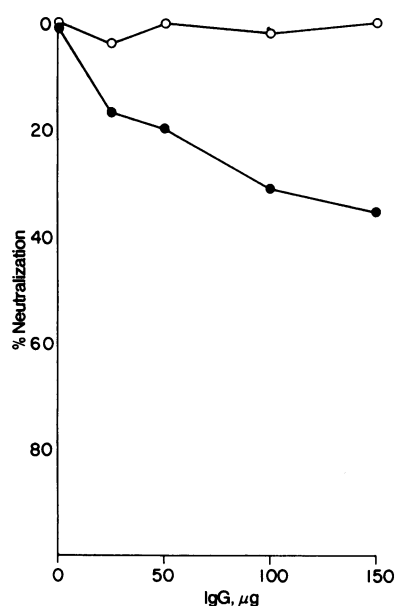


FIG. 2. Effect of anti-MPMV polymerase IgG on purified DNA polymerases from human breast cancer (●) and human leukemia (○) particles. Neutralization of DNA polymerase activities was performed as described in *Materials and Methods* and the legend to Fig. 1, with oligo(dG)-poly(rC) as template. Assays contained the indicated amounts of anti-MPMV DNA polymerase IgG, and controls containing comparable concentrations of normal (preimmunized) rabbit serum IgG were run to determine the percentage neutralization.

synthesis directed by synthetic templates (Figs. 1 and 2). As expected, all of the DNA polymerase activities described in Table 1 were sensitive to RNase and resistant to the presence of actinomycin D (100 μ g/ml) and distamycin (50 μ g/ml), features characteristic of RNA-directed DNA polymerase.

The data shown in Table 1 were obtained with the endogenous reactions of detergent-disrupted particles isolated from the indicated neoplastic tissues. For completeness, a similar comparison was carried out using the corresponding purified enzymes directed by oligo(dG)-poly(rC). An example of this approach is shown in Fig. 2, which describes the responses to anti-MPMV polymerase IgG of the reverse transcriptases purified from the particles prepared from breast cancers and from a chronic myelogenous leukemia spleen. It is clear that, over the whole concentration range of IgG examined, the leukemic reverse transcriptase was not significantly affected. In contrast, the anti-MPMV polymerase IgG suppressed the activity of the breast cancer reverse transcriptase at all concentrations tested, achieving a 37% inhibition at 150 μ g per reaction mixture.

Table 2 shows another aspect of the specificity of the interaction by testing the responses of normal cellular DNA polymerases to the anti-MPMV polymerase IgG. Neither

Table 2. Effect of anti-MPMV IgG on cellular and breast tumor particle polymerases

Source (enzyme)	With normal IgG	With anti-MPMV polymerase IgG	
	cpm	cpm	% of control
Breast tumor (reverse transcriptase)	1628	662	40.7
Breast tumor (DNA polymerase γ)	2166	2196	101.4
HeLa cell (DNA polymerase γ)	1865	2011	107.8
HeLa cell (DNA polymerase β)	1658	1819	109.7

The indicated DNA polymerases were assayed as described in *Materials and Methods*. The enzyme activity in the presence of normal rabbit IgG was taken as the control. Note that Table 1 compares normal IgG with bovine serum albumin. Each 100 μ l of assay mixture contained 75 μ g of IgG and 0.4 μ g of oligo(dT)-poly(rA); incubation was for 30 min at 37°.

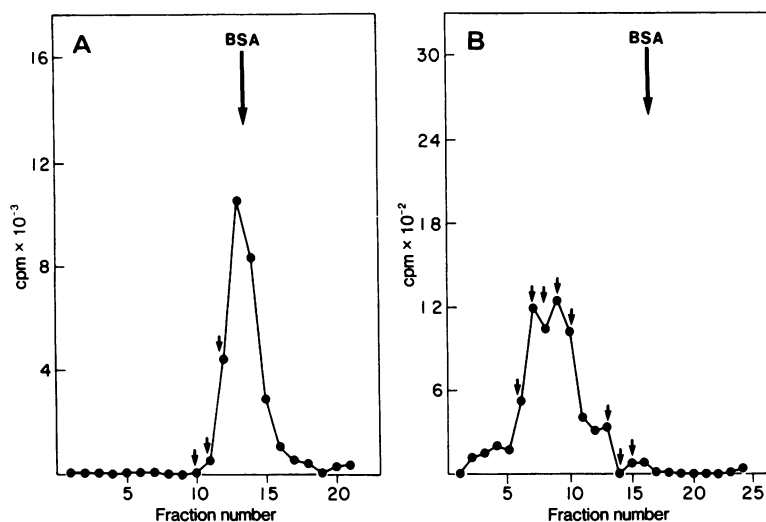


FIG. 3. Velocity sedimentation of MPMV DNA polymerase incubated with normal IgG and with anti-MPMV DNA polymerase IgG. Aliquots (250 μ l) containing purified MPMV DNA polymerase and 500 μ g of bovine serum albumin and either 150 μ g of normal rabbit IgG (A) or 150 μ g of anti-MPMV DNA polymerase IgG (B) were incubated for 15 min at 0 $^{\circ}$, layered on 10–30% glycerol gradient, and then centrifuged for 12 hr at 48,000 rpm in a Spinco SW50-1 rotor as described in *Materials and Methods*. After centrifugation, 21 to 24 fractions were collected; 25 μ l of every fraction was assayed for DNA polymerase activity in a standard reaction mixture, and 10 μ l from each fraction was tested for the presence of IgG by immunodiffusion assay with goat antiserum to rabbit IgG. The large arrow near the top of each panel indicates the position of bovine serum albumin (BSA) used as an external marker. The arrows close to the activity profiles indicate the fractions containing rabbit IgG as determined by immunodiffusion.

preparation of DNA polymerase γ , whether isolated from a breast tumor or from the HeLa cell strain, was detectably inhibited, and the same was true for DNA polymerase β . At the same level, the anti-MPMV polymerase IgG suppressed the breast cancer reverse transcriptase by 40%. We have carried out similar experiments (data not shown here) with the normal DNA polymerase α and again found no evidence of inhibition by the anti-MPMV polymerase IgG.

Demonstration by Sedimentation of Complexes between the Breast Cancer DNA Polymerase and the Anti-MPMV Polymerase IgG. Two issues made it desirable to see whether further evidence could be provided for the existence of physical complexes between the breast cancer reverse transcriptase and the anti-MPMV polymerase IgG. First, such evidence would add direct support to the conclusions derived from simple suppression of enzyme activity. Second, experiments along these

lines could identify the basis underlying the apparent inability of the anti-MPMV DNA polymerase IgG to achieve total neutralization of the breast cancer reverse transcriptase activity. Basically, two mechanisms can be offered to explain the incompleteness of the inhibition. One is that the enzyme preparation is heterogeneous and that only a subpopulation forms inactive complexes with the added IgG. The other is that the population of enzyme molecules is homogeneous in this respect and that all form complexes which can, however, express a fraction of the original activity. These two possibilities are readily distinguishable by a sedimentation analysis of the enzyme activity before and after reaction with the relevant IgG.

To monitor the effectiveness of this approach, a positive control experiment was carried out with the homologous system consisting of MPMV DNA polymerase and its antibody. A

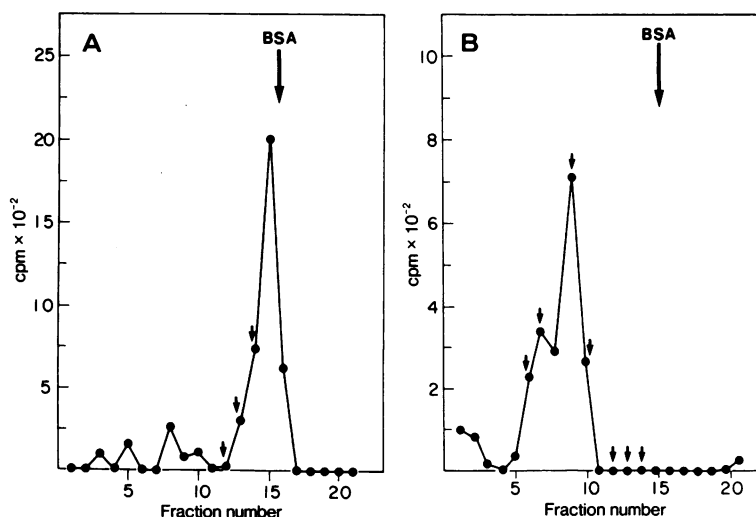


FIG. 4. Velocity sedimentation of DNA polymerase purified from human malignant breast tumor particles incubated with normal IgG and with anti-MPMV DNA polymerase IgG. Aliquots (250 μ l) containing purified human breast tumor DNA polymerase and either 150 μ g of normal rabbit IgG (A) or 150 μ g of anti-MPMV DNA polymerase IgG (B) were incubated and subsequently treated as described in *Materials and Methods* and in the legend of Fig. 3. The arrow at the top of each panel indicates the position of bovine serum albumin (BSA), the external marker. The arrows close to the activity profiles indicate the fractions containing rabbit IgG as determined by immunodiffusion.

negative control was included with normal (preimmunized) IgG from the same rabbit. The 250- μ l reaction mixture containing enzyme and 150 μ g of the indicated IgG was incubated at 4° for 15 min as described in *Materials and Methods*. The mixture was then layered on a 10–30% gradient and centrifuged at 48,000 rpm in a Spinco SW50-1 rotor for 12 hr at 1°. Fractions were collected from the bottom of the tube and assayed for reverse transcriptase and for the presence of IgG by immunodiffusion. As shown in Fig. 3A, incubation with normal IgG did not change the position (tube 13) of the peak of MPMV reverse transcriptase activity with respect to the external marker, bovine serum albumin. The enzyme still sedimented at a velocity corresponding to a molecular weight of 70,000. In this same gradient, the IgG was located in tubes 10, 11, and 12. Incubation of the polymerase with 150 μ g of anti-MPMV polymerase resulted in an 80% loss of enzyme activity and a markedly different sedimentation pattern of the residual activity. No enzyme was detectable at the original position close to bovine serum albumin, all of it appearing as complexes sedimenting faster than free enzyme or IgG. The IgG was detected by immunodiffusion in fractions 13, 14, and 15 as well as in fractions 6 through 10 which encompass the peak of polymerase activity.

A similar situation occurred in the experiments, described in Fig. 4, with the reverse transcriptase purified from human breast cancer particles. Incubation with normal IgG (Fig. 4A) left the human enzyme in its usual position (tube 15), within one tube of the marker, and the IgG was found by immunodiffusion in tubes 12 to 14. However, reaction with anti-MPMV polymerase IgG resulted in a 45% loss of activity and shifted the residue down the tube as fast-moving complexes found in fractions 6 through 10 in which IgG also was detected by immunodiffusion (Fig. 4B). IgG also was found in its original position (fractions 12 through 14).

It is evident from the results described in Figs. 3 and 4 that neither the MPMV reverse transcriptase nor the one isolated from human breast cancer particles contains a significant proportion of molecules unable to complex with anti-MPMV polymerase IgG. The fact that the enzyme-IgG complexes can express some activity is not a new phenomenon. Indeed, in some reported instances such complexes are fully active (21) and this property was used to develop a convenient immunoprecipitation of specific ribosomes carrying newly synthesized chains of specific proteins (22).

DISCUSSION

The experiments described here show that anti-MPMV DNA polymerase IgG, when present in excess, can completely complex with and partially inhibit the reverse transcriptase isolated from human breast cancer particles. The specificity of the inhibition is supported by the inability of this same antibody to affect the activities of normal cellular DNA polymerases or of various reverse transcriptases from animal oncornaviruses. Further, normal IgG obtained from the same rabbit prior to immunization does not complex with or inhibit either the MPMV or the human breast cancer reverse transcriptase.

Aside from its etiologic interest, the immunologic cross-reactivity between the reverse transcriptases of MPMV and of the human breast cancer particles has an implication of more immediate import. It suggests the existence of a novel pathway for examining human breast cancer by procedures of potential clinical usefulness. MPMV can be produced in tissue culture in yields adequate for purification of its enzyme and other protein components. These can in turn be used to generate

antisera for use as specific detecting reagents in immunofluorescent and immunoperoxidase staining of frozen sections as possible diagnostic aids for surgical pathologists. Of even wider interest is the possible development of radioimmunoassays for the systemic detection of immunologically related protein in the plasma and other body fluids of patients with breast cancer.

This latter approach is made particularly attractive by the recent demonstration of its feasibility in the murine mammary tumor model. It has been shown (23–25) that the plasma level of gp52, a protein of the mouse mammary tumor virus, is an excellent systemic indicator of the presence and extent of mammary neoplasia.

We would like to express our appreciation to Ms. I. Doneva for her excellent technical assistance. This investigation was supported by Grant CA-02332 and Contract NO1-6-1010 awarded by the National Cancer Institute.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

1. Axel, R., Schlom, J. & Spiegelman, S. (1972) *Nature* **235**, 32–36.
2. Vaidya, A. B., Black, M. M., Dion, A. S. & Moore, D. H. (1974) *Nature* **249**, 565–567.
3. Colcher, D., Spiegelman, S. & Schlom, J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4975–4979.
4. Chopra, H. C. & Mason, M. M. (1970) *Cancer Res.* **30**, 2081–2086.
5. Schlom, J. & Spiegelman, S. (1971) *Science* **174**, 840–843.
6. Axel, R., Gulati, S. C. & Spiegelman, S. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3133–3137.
7. Michalides, R., Spiegelman, S. & Schlom, J. (1975) *Cancer Res.* **35**, 1003–1008.
8. Ohno, T., Sweet, R. W., Hu, R., DeJek, D. & Spiegelman, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 764–768.
9. Parks, W. P., Scolnick, E. M., Ross, J., Todaro, G. J. & Aaronson, S. A. (1972) *J. Virol.* **9**, 110–115.
10. Nowinski, R. C., Watson, K. F., Yaniv, A. & Spiegelman, S. (1972) *J. Virol.* **10**, 959–964.
11. Scolnick, E., Parks, W. & Todaro, G. (1972) *Science* **117**, 1119–1121.
12. Oroszlan, S., Hatanaka, M., Gilden, R. & Huebner, R. (1971) *J. Virol.* **8**, 816–818.
13. Scolnick, E., Parks, W., Todaro, G. & Aaronson, S. (1972) *Nature New Biol.* **235**, 35–40.
14. Long, C., Sacho, R., Nowell, J., Huebner, R., Hatanaka, M. & Gilden, R. (1973) *Nature New Biol.* **241**, 143–149.
15. Yaniv, A., Ohno, T., Kacian, D., Colcher, D., Witkin, S., Schlom, J. & Spiegelman, S. (1974) *Virology* **59**, 335–338.
16. Todaro, G. & Gallo, R. (1973) *Nature* **244**, 206–209.
17. Gallagher, R., Todaro, G., Smith, R., Livingston, D. & Gallo, R. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1309–1313.
18. Schlom, J. & Spiegelman, S. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 1613–1617.
19. Witkin, S., Ohno, T. & Spiegelman, S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4133–4136.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
21. Rotman, B. & Spiegelman, S. (1954) *J. Bacteriol.* **68**, 419–429.
22. Cowie, D. B., Spiegelman, S., Roberts, R. B. & Duerksen, J. D. (1961) *Proc. Natl. Acad. Sci. USA* **47**, 114–122.
23. Ritzi, E., Baldi, A. & Spiegelman, S. (1976) *Virology* **75**, 188–197.
24. Ritzi, E., Martin, D. S., Stolfi, R. L. & Spiegelman, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4190–4194.
25. Ritzi, E., Martin, D. S., Stolfi, R. L. & Spiegelman, S. (1977) *J. Exp. Med.*, in press.