Antitumor Activity of Silica Gel PF 254 Eluate

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SUMMARY

Tumor growth in NMRI mice infected neonatally with the Moloney murine sarcoma virus was effectively inhibited by an aqueous eluate from the silica gel "Kieselgel PF 254." This inhibitory effect was observed whether the silica gel eluate was injected 12 hr before virus inoculation or mixed directly with the virus inoculum. Upon mixing with the Moloney murine sarcoma virus, the silica gel eluate was barely effective if the virus-eluate mixture was incubated for only 1 min (at 25°C) but significantly more effective if the mixture was incubated for 5 min (or more) (at 25°C), suggesting that the antitumor activity of the eluate was due to a direct inactivating effect on the virus. This virus-inactivating effect was equally well expressed in immunocompetent (NMRI) as in immunoincompetent (genetically thymusless nude) mice. The postulated virucidal effect of silica gel eluate appeared to be limited to oncogenic RNA viruses; the eluate did not alter the infectivity of other viruses such as vaccinia, herpes simplex, Newcastle disease, vesicular stomatitis, and polio. The Moloney murine sarcoma virus-inhibiting effect of the silica gel eluate might have been related to a stimulatory effect on the RNA-dependent DNA polymerase activity of oncogenic RNA viruses, as a markedly increased DNA synthesis was noted in an in vitro DNA polymerase assay with the Moloney murine leukemia virus. However, the silica gel eluate did not affect the integrity of the Moloney murine leukemia virus, as assayed by isopycnic centrifugation of treated and untreated virus particles in a sucrose density gradient.

INTRODUCTION

Newborn mice inoculated with MSV\(^2\) rapidly develop tumors at the site of the inoculation (20, 21). Pathologically, the tumors have been identified as rhabdomyosarcomas (21). They are thought to evolve by evoked proliferation, that is, continuous recruitment of normal contiguous cells by new viral infection, rather than by clonal proliferation of already infected cells. MSV-induced tumors in newborn mice rarely regress but grow progressively and kill the host; tumors induced in adult mice ultimately regress, unless the hosts have been rendered immunologically hyporesponsive, e.g., by X-irradiation, cortisone treatment, or cyclophosphamide treatment (8–11, 17, 26).

By virtue of some unique characteristics (short latency period to tumor formation, regularity of tumor induction) the MSV-induced tumor model appeared especially suited for evaluating potential antiviral agents. It has been used previously to assess the antitumor activity of interferon (2, 5, 23) and interferon inducers such as poly(I)-poly(C) (5, 7, 23–25, 31) and chlorite-oxidized oxyamylose (6).

The present report describes the antitumor activity of Silica Gel PF 254 eluate. This eluate was found to inhibit MSV-induced tumor formation in newborn mice, whether the compound was administered prior to or together with the virus challenge. The antitumor activity of the eluate could be attributed to a specific inactivation of the Moloney sarcoma virus. Experiments aimed at delineating the mechanism of the direct virucidal effect of the silica gel eluate are also dealt with in the present report.

MATERIALS AND METHODS

Animals. Randomly bred NMRI mice used in our experiments were obtained from the Animal Production Section of the University of Leuven. Nude mice (also obtained from the Animal Production Section of the University of Leuven) were bred essentially as described previously (3). Details of the breeding will be described separately (E. De Clercq, W. E. Stewart II, and P. De Somer, manuscript in preparation). Experiments were done with both homozygous nu/nu and heterozygous nu/NMRI mice. The animals were kept in ordinary housing conditions.

Cell Cultures. Primary rabbit kidney cells as well as continuous cell lines of baby hamster kidney and HeLa cells were used for assaying the infectivity of vaccinia virus, herpes simplex virus, VSV, NDV, and poliovirus. The cells were grown to confluency in minimal (Eagle’s) essential medium supplemented with 10% inactivated calf serum. Upon virus inoculation, only 3% calf serum was added.

Viruses. M-MSV was prepared as described previously (5). The stock contained a 50% tumor-inducing dose of 10\(^5\) / 0.025 ml when titrated in 2-day-old NMRI mice. For this titration, serial dilutions of the virus stock were prepared in a solution containing 0.5 M sucrose and 0.00075 M Tris-HCl (pH 7.2); the samples were then injected i.m. (right thigh) at approximately 48 hr after birth (0.025 ml/mouse). For the experiments reported in this paper, virus stock dilutions were either made in 0.5 M sucrose:0.00075 M Tris-
HCl (pH 7.2) and injected at 0.025 ml/mouse (Charts 1 and 2) or made in silica gel eluate or phosphate-buffered saline (Charts 3 to 6) and injected at 0.033 ml/mouse.

M-MLV was supplied by Electro-Nucleonics Laboratories (Bethesda, Md.) (Catalog No. 1021). The virus stock, purified by double density gradient zonal centrifugation (30) contained 2 to 3 x 10^11 particles/ml of 0.1 m NaCl: 0.01 m Tris-HCl (pH 7): 0.001 m EDTA. It was stored at -70° until used. Prior to use, 8% of glycerol was added to the sample; the virus could be stored at 4° in the presence of 8% glycerol for at least 1 month without loss of DNA polymerase activity.

Vaccinia virus, herpes simplex virus, and poliovirus were propagated in HeLa cells. VSV was maintained in primary mouse embryo fibroblasts, whereas NDV was grown in the chorioallantoic cavity of chick embryos.

Silica Gel Eluate. The silica gel eluate was prepared by mixing 20 g Silica Gel PF 254 (Kieselgel PF 254 with CaSO4), for preparative thin-layer chromatography, purchased from E. Merck AG, Darmstadt, Germany with 50 ml phosphate-buffered saline for 2 hr at room temperature. The sample was then centrifuged for 15 min at 3000 rpm and the supernatant was filtered through a Millipore filter (pore size, 450 nm) (Millipore Company, Bedford, Mass.). The filtrate (designated "silica gel eluate") was stored at 4° until used.

Chemicals. dTTP-^3H (11.3 or 23.9 Ci/mmole, depending on the batch) (The Radiochemical Center, Amersham, England) was purchased from the C. E. N. Radioisotopes Department, Mol, Belgium. The unlabeled deoxyribonucleoside triphosphates dATP, dCTP, and dGTP, as well as calf thymus DNA, dithiothreitol, and pancreatic RNase (bovine, 5 times crystallized), were obtained from Sigma Chemical Company (St. Louis, Mo.). NaH2PO4 and Na4P2O7 were both purchased from E. Merck. Omnifluor scintillation fluid was a product of NEN Chemicals, Frankfurt-on-Main, Germany. Poly(C)-^3H (44.8 mCi/mmol phosphorus, 8.95 μg/mCi), dispensed in 50% ethanol, was obtained from Miles Laboratories, Elkhart, Ind.; the polymer was lyophilized, dissolved in phosphate-buffered saline at 10 μg/ml, and stored at -20°.

RNA-dependent DNA Polymerase Assay. A standard reaction mixture (250 μl) contained 40 mM Tris-HCl (pH 7.8); 50 mM NaCl; 4 mM MnCl2; 1.6 mM dithiothreitol; 0.0125% Triton X-100; 0.64 mM each of unlabeled dATP, dCTP, and dGTP; 0.0035 mM dTTP-^3H. For further details, see the legends to Charts 7 and 8. Special precautions were taken to diminish nonincorporated (trapped) counts in the RNA-dependent DNA polymerase assay product (4). Each sample was treated with 1 ml of calf thymus DNA (12 μg/ml) in saturated phosphate (mixture of equal volumes of saturated NaH2PO4 and saturated Na2P2O7) at 0°, and then 1 ml of cold TCA (16% in saturated phosphate) was added. After 20 min at 0°, the precipitates were collected on glass fiber discs (Whatman GF/C, 2.4 cm in diameter; presoaked in saturated phosphate) and mounted on a porcelain Hirsch funnel. The filters were washed with 40 ml cold TCA (8% in saturated phosphate) and then rinsed with cold absolute ethanol. The filters were dried and counted in 5 ml of toluene: PPO scintillation fluid (Omnifluor) in a Packard Tri-Carb liquid scintillation spectrometer.

Degradation of poly(C)-^3H by Pancreatic RNase. The rate of degradation of poly(C)-^3H by pancreatic RNase was monitored by measuring acid-insoluble radioactivity in 0.2 ml samples withdrawn at different times after exposure of the polynucleotide to the enzyme (see legend to Chart 9). To measure acid-insoluble radioactivity, 0.2 ml of 0.1% yeast RNA and 1.0 ml of 5% TCA were added to 1.0-ml samples at 0°. The precipitates were collected by filtration on Gelman type A glass fiber discs (diameter, 2.5 cm) and washed with cold TCA (5%). The filters were dried and counted as outlined above (see "RNA-dependent DNA Polymerase Assay").

RESULTS

Influence of Silica Gel Eluate on Tumor Development in NMRI Mice Neonatally Infected with M-MSV. (Eluate Injected i.p. at 12 hr before Virus Challenge). Three silica gel eluates prepared from 3 different PF 254 thin-layer plates were all effective in inhibiting the development of MSV tumors when injected i.p. at 12 hr before virus challenge (Charts 1 and 2). Single doses of only 0.1 ml of the eluates were administered. The M-MSV stock was used at a 10^-2.6 dilution prepared in 0.5 M sucrose: 0.00075 M Tris-HCl (pH 7.2) and injected i.m. at 0.025 ml/mouse. The silica gel eluates reduced both tumor incidence (Chart 1) and mortality due to tumor formation (Chart 2).

Influence of Silica Gel Eluate on Tumor Development in NMRI Mice Neonatally Infected with M-MSV (Virus Mixed with the Eluate and Incubated at 25° for 1 hr before Inoculation). To investigate whether the silica gel eluate may have a direct inactivating effect on the virus, dilutions of the M-MSV stock were directly prepared in the eluate. The tumorigenicity of these dilutions was compared with similar dilutions (10^-1.3, 10^-1.78, and 10^-2.7) prepared in phosphate-buffered saline. All samples were first incubated

![Chart 1. Effect of single doses of 3 different silica gel eluate preparations on tumor incidence in NMRI mice infected neonatally with M-MSV (10^-2.4 dilution). One-tenth ml of the eluate was injected i.p. at 12 hr before virus challenge. Twenty mice per group.](image-url)
Antitumor Activity of Silica Gel PF 254 Eluate in injected i.m. at 0.033 ml/mouse into homozygous nu/nu mice and their heterozygous nu/NHRI littermates. As shown in Chart 6, tumor incidence and death due to tumor growth were significantly reduced in those mice that received virus prepared in silica gel eluate.

**Influence of Silica Gel Eluate on the Infectivity of Viruses Other Than M-MSV.** Additional experiments were designed to find out whether the direct virucidal effect of the silica gel eluate was limited to oncogenic RNA viruses such as M-MSV or encompassed other viruses. Representatives of the major virus groups (DNA, RNA, enveloped, and nonenveloped) were tested: vaccinia virus; herpes simplex virus; NDV; VSV; and poliovirus. Exposure of these viruses

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**Chart 2.** Effect of single doses of 3 different silica gel eluate preparations on mortality in NMRI mice infected neonatally with M-MSV (10⁻² dilution). One-tenth ml of the eluate was injected i.p. at 12 hr before virus challenge. Twenty mice per group.

for 1 hr at 25° and then injected i.m. at 0.033 ml/mouse. The results depicted in Charts 3 and 4 clearly show that tumor development (as measured by both tumor incidence and death due to tumor growth) was significantly inhibited with all M-MSV samples prepared in silica gel eluate.

**Influence of Silica Gel Eluate on Tumor Development in NMRI Mice Neonatally Infected with M-MSV (Virus Mixed with the Eluate and Incubated at 25° for Varying Periods of Time before Inoculation).** If the antitumor activity of silica gel eluate is due to a direct inactivating effect on M-MSV, one might expect a positive correlation between the extent of virus inactivation and the time of exposure of the virus to the silica gel eluate. Therefore, a 10⁻¹.₇₈ dilution of M-MSV was prepared in silica gel eluate and incubated at 25° for different times (1, 5, 20, or 60 min). The samples were then injected i.m. at 0.033 ml/mouse. Data illustrating the kinetics of inactivation of M-MSV are presented in Chart 5. A significant reduction in tumor appearance and mortality was recorded with all samples that had been incubated in the presence of the silica gel eluate for 5 min or longer. A slight delay in tumor appearance but no reduction in the mortality rate occurred if the virus had been exposed to the eluate for only 1 min. No differences were noted among control samples that had been incubated for 1, 5, 20, or 60 min (only the 60-min sample is presented in Chart 5). Thus, the inhibitory effect of the silica gel eluate on the infectivity of M-MSV depended on the time of contact between the virus and the eluate, as could be expected if this inhibitory effect was due to a direct inactivation of the virus.

**Influence of the Silica Gel Eluate on Tumor Development in Nude Mice Neonatally Infected with M-MSV (Virus Mixed with the Eluate and Incubated at 25° for 1 hr before Inoculation).** If the antitumor activity of the silica gel eluate is due to a direct virucidal effect on the virus and not to host-mediated defense mechanisms, it should also be effective in the genetically thymusless nude mice in which immunological competence is significantly impaired (3). To test this hypothesis, suspensions of M-MSV stock diluted 10⁻² in silica gel eluate or phosphate-buffered saline were

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**Chart 3.** Tumor incidence in NMRI mice infected neonatally with M-MSV diluted 10⁻¹.₄ (A), 10⁻¹.₇₈ (B), or 10⁻¹.₉ (C), in either silica gel eluate or phosphate-buffered saline (control). The samples were incubated for 1 hr at 25° and then injected i.m. at 0.033 ml/mouse. Twenty mice per group.
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Chart 4. Mortality due to tumor development in NMRI mice infected neonatally with M-MSV diluted 10⁻¹·₂ (A), 10⁻¹·₄ (B), or 10⁻¹·₆ (C), in either silica gel eluate or phosphate-buffered saline (control). The samples were incubated for 1 hr at 25° and then injected i.m. at 0.033 ml/mouse. Twenty mice per group.

to the silica gel eluate for 1 hr at 25° did not affect their infectivity (Table 1) (as compared to the infectivity recorded with control virus samples exposed to phosphate-buffered saline). Thus, the spectrum of activity of the silica gel eluate was restricted to oncogenic RNA viruses such as M-MSV.

Influence of Silica Gel Eluate on the RNA-dependent DNA Polymerase Activity of M-MLV. Because only M-MSV appeared to be inactivated by the silica gel eluate, the inactivating effect of the eluate could be specifically related to an impairment of the RNA-dependent DNA polymerase activity in oncogenic RNA virions. To test this possibility, the silica gel eluate was added to a standard reaction mixture for assaying the DNA polymerase activity in RNA tumor viruses (4). Highly purified M-MLV, a virus closely related to M-MSV, provided both the endogenous RNA template and the RNA-dependent DNA polymerase. DNA synthesis, as monitored by dTMP-³H incorporation, was markedly increased in the presence of the silica gel eluate (Chart 4). In the absence of the eluate, dTMP-³H incorporation leveled off after 20 min of incubation, while assay mixtures containing the eluate showed almost linear kinetics for up to 60 min.

To prove that the DNA polymerase activity detected in M-MLV was really RNA dependent, M-MLV (20 μl of the stock preparation) was first incubated with pancreatic RNase (20 μg/20 μl of 0.04 M Tris-HCl (pH 7.8)) at 22° for 30 min, before the components of the reaction mixture (including 20 μl of either silica gel eluate or phosphate-buffered saline) were added. Pretreatment of the virions with RNase caused a 50% inhibition of the DNA polymerase activity at 30 min and 70 to 80% inhibition at 120 min, no matter whether silica gel eluate or phosphate-buffered saline was included in the reaction mixture. It may be contended, therefore, that the endogenous viral RNA is the template for the DNA polymerase reaction. That pretreatment of the virions with RNase did not prevent all incor-

Chart 5. Tumor incidence (A) and mortality due to tumor development (B) in NMRI mice infected neonatally with M-MSV diluted 10⁻¹·₂, in either silica gel eluate or phosphate-buffered saline (control). The control sample was incubated at 25° for 1 hr. The samples made up in silica gel eluate were incubated at 25° for different times ranging from 1 min to 1 hr. The samples were injected i.m. at 0.033 ml/mouse. Twenty mice per group.
Chart 6. Tumor incidence (A) and mortality due to tumor development (B) in nude (nu/nu) mice and nu/NMRI hybrid mice infected neonatally with M-MSV diluted $10^{-2}$, in either silica gel eluate or phosphate-buffered saline (control). The samples were incubated for 1 hr at 25°C and then injected i.m. at 0.033 ml/mouse. Ten to 20 mice per group.

Table 1

<table>
<thead>
<tr>
<th>Virus group</th>
<th>Virus type</th>
<th>Control</th>
<th>Eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poxivirus</td>
<td>Vaccinia</td>
<td>$4.5 \times 10^4$</td>
<td>$4.0 \times 10^4$</td>
</tr>
<tr>
<td>Herpesvirus</td>
<td>Herpes simplex</td>
<td>$1.0 \times 10^6$</td>
<td>$1.0 \times 10^6$</td>
</tr>
<tr>
<td>Paramyxovirus</td>
<td>NDV</td>
<td>$1.0 \times 10^6$</td>
<td>$1.0 \times 10^6$</td>
</tr>
<tr>
<td>Rhabdovirus</td>
<td>VSV</td>
<td>$8.0 \times 10^4$</td>
<td>$7.0 \times 10^4$</td>
</tr>
<tr>
<td>Picornavirus</td>
<td>Polio</td>
<td>$1.0 \times 10^4$</td>
<td>$1.0 \times 10^4$</td>
</tr>
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*Expressed in plaque-forming units/ml for vaccinia, herpes simplex, and VSV and in tissue culture-infecting dose for 50% of the cultures per ml for NDV and polio.

Incorporation of dTMP-$^3$H suggests that the viral RNA is at least partially masked by protein (29).

**Influence of Silica Gel Eluate on the Integrity of M-MLV.** To investigate whether treatment with silica gel eluate resulted in a disruption and consequent loss of a significant portion of the virion, treated and untreated M-MLV was subjected to isopycnic centrifugation in a 15 to 60% sucrose density gradient. Fractions of the gradient were assayed for RNA-dependent DNA polymerase activity. There was no difference in the localization of DNA polymerase activity between the treated and untreated samples (Chart 8). In both cases the DNA polymerase activity peaked at a density of 1.145 g/ml, that is, at a slightly lower density level than generally obtained with oncogenic RNA viroins (1, 16).

**Influence of Silica Gel Eluate on the Activity of Pancreatic RNase.** To test the possibility that part of the effects of the silica gel eluate, and, more specifically, its enhancing effect on the RNA-dependent DNA polymerase activity were due to an inhibition of RNase, silica gel eluate was added to a reaction mixture containing 0.2 μl of poly(C)-$^3$H per ml and either 0.01 or 0.001 μg of pan-
RNA-dependent DNA polymerase activity was determined by mixing 1/10 ml aliquots of the M-MLV preparation with either poly(C)-3H or phosphate-buffered saline (control) and incubating at 37°C for 1 hr. The preparations were then layered on top of a linear gradient of 15 to 60% (w/v) sucrose in 0.1 M Tris-HCl (pH 8.3): 0.12 M NaCl. After centrifugation for 90 min at 40,000 rpm in an MSE 6 x 5 ml titanium swing-out rotor, 0.2-ml fractions of the gradient were collected from the top of the tubes using Model 640 Isco density gradient fractionator. RNA-dependent DNA polymerase activity was determined in 0.05-ml portions of each fraction incubated for 60 min at 37°C in a standard reaction mixture (see “Materials and Methods”). The acid-precipitable radioactivity was then collected and counted. •, virus treated with silica gel eluate; O, control virus. Numbers along the ordinate should be multiplied by 10² to obtain the correct values.

The antitumor activity of the silica gel eluate is probably due to a direct virucidal effect on the virus, as suggested by (a) the loss of tumorigenicity of the virus and (b) the increase of its reverse transcriptase activity upon mixing with the eluate. No such loss of tumorigenicity or increase in reverse transcriptase activity was observed upon mixing the virus with comparable doses of (endon)ucleases.

Although the antitumor effects of poly(I)·poly(C) (5), interferon (5), and silica gel eluate (administered 12 hr before M-MSV challenge; Charts 1 and 2) are quantitatively similar, the mechanisms by which they achieve these effects appear to differ markedly. The antitumor activity of the silica gel eluate probably due to a direct virucidal effect on the virus, as suggested by (a) the loss of tumorigenicity of the virus and (b) the increase of its reverse transcriptase activity upon mixing with the eluate. No such loss of tumorigenicity or increase in reverse transcriptase activity was observed upon mixing the virus with comparable doses of either poly(I)·poly(C) or interferon.

The antitumor activity of interferon and poly(I)·poly(C) is clearly mediated by host defense mechanisms since their antitumor effects largely depend on the genetic and/or immune status of the animal. Thus, poly(I)·poly(C) (and interferon) inhibited MSV-induced tumor development in NMRI mice (5) but rather enhanced the growth of MSV-induced tumors in many other mouse strains [e.g., AL/N mice (12–14)]. There are also preliminary indications that interferon and poly(I)·poly(C) stimulate MSV-induced tumor growth in nude mice and nude/NMRI hybrids (E. De Clercq, W. E. Stewart II, and P. De Somer, manuscript in preparation). Silica gel eluate inhibited MSV-infection.

**DISCUSSION**

Eluates from Silica Gel PF 254 thin-layer plates were shown to inhibit Moloney sarcoma virus-induced tumor formation in newborn mice. The extent of the antitumor activity obtained with single doses of the eluates injected i.p. 12 hr before virus challenge (Charts 1 and 2) was in the range of the antitumor activity observed with poly(I)·poly(C) and interferon in similar experiments (5). In the latter experiments, poly(I)·poly(C) was injected i.p. at 25 (or 5) µg/

Chart 8. Isopycnic sucrose gradient analysis of M-MLV treated with silica gel eluate, as monitored by RNA-dependent DNA polymerase activity. One-tenth-ml aliquots of the M-MLV preparation were mixed with 0.1 ml of silica gel eluate or phosphate-buffered saline (control) and incubated at 37°C for 1 hr. The preparations were then layered on top of 4.6 ml of a linear gradient of 15 to 60% (w/v) sucrose in 0.1 M Tris-HCl (pH 8.3): 0.12 M NaCl. After centrifugation for 90 min at 40,000 rpm in an MSE 6 x 5 ml titanium swing-out rotor, 0.2-ml fractions of the gradient were collected from the top of the tubes using Model 640 Isco density gradient fractionator. RNA-dependent DNA polymerase activity was determined in 0.05-ml portions of each fraction incubated for 60 min at 37°C in a standard reaction mixture (see “Materials and Methods”). The acid-precipitable radioactivity was then collected and counted. •, virus treated with silica gel eluate; O, control virus. Numbers along the ordinate should be multiplied by 10² to obtain the correct values.

Chart 9. Rate of degradation of poly(C)-3H by pancreatic RNase in the presence of silica gel eluate. Poly(C)-3H (final concentration 0.2 µg/ml) was incubated with pancreatic RNase [final concentration, 0.01 µg/ml (A) or 0.001 µg/ml (B)] for different times at 37°C in phosphate-buffered saline (total volume of reaction mixture, 2 ml). Silica gel eluate was included in some reaction mixtures at a ratio of 0.16 ml/total volume (2 ml); this ratio corresponds to the concentration of silica gel eluate used in the RNA-dependent DNA polymerase assay (20 µl/250 µl). At the indicated times 0.2-ml samples of the reaction mixtures were withdrawn for analysis of TCA-precipitable radioactivity (see “Materials and Methods”). Δ, poly(C)-3H; O, poly(C)-3H + RNase; •, poly(C)-3H + RNase + eluate. Numbers along the ordinate should be multiplied by 10² to obtain the correct values.

Mouse 12 hr before virus challenge and mouse interferon was administered i.p. in 3 doses of 200 units each at 12, 8, and 4 hr before virus challenge; the virus stock was injected i.m. at a 10⁻² dilution (0.025 ml/mouse), thus in identical conditions as those used in the present study (Charts 1 and 2).

Although the antitumor effects of poly(I)·poly(C) (5), interferon (5), and silica gel eluate (administered 12 hr before M-MSV challenge; Charts 1 and 2) are quantitatively similar, the mechanisms by which they achieve these effects appear to differ markedly. The antitumor activity of the silica gel eluate is probably due to a direct virucidal effect on the virus, as suggested by (a) the loss of tumorigenicity of the virus and (b) the increase of its reverse transcriptase activity upon mixing with the eluate. No such loss of tumorigenicity or increase in reverse transcriptase activity was observed upon mixing the virus with comparable doses of either poly(I)·poly(C) or interferon.

The antitumor activity of interferon and poly(I)·poly(C) is clearly mediated by host defense mechanisms since their antitumor effects largely depend on the genetic and/or immune status of the animal. Thus, poly(I)·poly(C) (and interferon) inhibited MSV-induced tumor development in NMRI mice (5) but rather enhanced the growth of MSV-induced tumors in many other mouse strains [e.g., AL/N mice (12–14)]. There are also preliminary indications that interferon and poly(I)·poly(C) stimulate MSV-induced tumor growth in nude mice and nude/NMRI hybrids (E. De Clercq, W. E. Stewart II, and P. De Somer, manuscript in preparation). Silica gel eluate inhibited MSV-infection.

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3 E. De Clercq, unpublished observations, 1972.
duced tumor growth in both NMRI, nude and nude/ NMRI hybrids. This is in concert with the proposed mode of action of the eluate, that is a direct inactivation of the virus.

Attempts to transfer the antitumor activity of the silica gel eluate by either serum or peritoneal cells failed. In these experiments serum and peritoneal cells (primarily macrophages) were collected from adult mice 12 hr after i.p. administration of 1.0 ml of the eluate. The serum and peritoneal macrophages were administered to baby mice (at a ratio of 1 adult mouse per 5 baby mice) simultaneously with the i.m. inoculation M-MSV (diluted at 10^{-2.2}, 0.025 ml/mouse). The effects of serum and of peritoneal macrophages collected from mice treated with the silica gel eluate did not differ significantly from the effects obtained with control serum and control macrophages, suggesting that the antitumor activity of the silica gel eluate could not be transferred by either serum or cells.

The inhibitory effect of silica gel eluate on M-MSV induced tumor growth is most likely due to a direct inactivation of the virus, even when the eluate is administered before virus challenge. This direct virucidal effect might be related to a stimulation of the RNA-dependent DNA polymerase in the virion. How may the silica gel eluate stimulate the DNA polymerase activity? Possibly, it is by inhibition of other enzymes present in the virions of RNA tumor viruses (e.g., endo- and exonucleases) (18, 19, 22). However, it is difficult to visualize how this explanation would fit in with the suppressed tumor growth observed in vivo (Charts 1 to 6). In fact, the silica gel eluate was tested for its effect on the rate of degradation of poly(C)-3H in the presence of pancreatic RNase, and, although minute amounts of RNase were included in the reaction mixture, silica gel eluate failed to inhibit the enzymatic activity (Chart 9).

Alternatively, the stimulatory effect of the eluate of the RNA-dependent DNA polymerase activity may be the consequence of a disruption of the virions. Thus, the effect may be very much detergent-like, for it has been well established that detergents (e.g., Nonidet P-40, Triton X-100, Tween 40, Tween 80, and ether) should be added to the RNA polymerase assay mixtures to uncover DNA polymerase activity (I, 4, 15, 16, 28, 29). However, no DNA polymerase activity was observed if Triton X-100 was substituted for by silica gel eluate in our reaction mixture (data not shown), suggesting that the eluate cannot take over the role of the detergent. However, the eluate may complement the role of Triton X-100, e.g., by removing lipids or lipoproteins from the surface of the virion, thereby stimulating the penetration of the substrates (dATP, dCTP, dGTP, and dTTP) into the core of the virion, and/or favoring the release of finished DNA product. As noted with the silica gel eluate (Charts 3 and 4), Triton X-100 inhibited M-MSV-induced tumor growth when present in the virus inoculum (at 0.1 or 0.01%). However, in marked contrast with the silica gel eluate (Charts 1 and 2), Triton X-100 failed to suppress tumor growth when injected prior to or simultaneously with but at a distant site from the virus challenge.

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