INHIBITION OF TUMOR TRANSPLANTATION BY DNA*

Richard M. Halpern, B. Cutting Halpern, G. Ciliv
and Roberts A. Smith
Division of Biochemistry, Department of Chemistry
and Department of Medicine,
University of California, Los Angeles, California 90024

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During an investigation primarily directed toward isolating an infectious nucleic acid from non-viral neoplasms of rodents, we have repeatedly isolated a DNA which consistently and paradoxically inhibits the transplantability of these tumors.

The transplantation of normal tissue invariably leads to an unsustained local inflammatory swelling or tumor but never to a persistent growth of the implanted cells, unless the host is prepared with cortisone and or other immunosuppressive techniques, or the cells are injected into special sites (Corille et al. 1957). The situation is vastly different when one transplants Walker carcinosarcoma (W-256) or Crocker sarcoma (S-180) into inbred rats and mice respectively. In our hands, 2×10^5 neoplastic cells implanted subcutaneously is an LD_{50} dose.

Neoplastic cell suspensions were prepared from aseptically excised 7 to 30 day old tumors. Usually 10g. of tumor were diced and gently homogenized in 100 ml of Robinson's (1949) buffer containing 1 mg/ml of penicillin G. The suspension was filtered through several layers of cheesecloth and centrifuged at 120 g for 5 min. at 0°C. After repeated washing and centrifugation the final cell suspension in Robinson's buffer was counted in a hemocytometer and stored at 4°C until used the same day.

DNA preparations were obtained from W-256 and S-180 neoplasms as well as from the liver of healthy rats using the procedure of Kay, Simmons and Dounce (1952). The first ethanol precipitate obtained by this technique was stored under ethanol at -10°C. When used it was pressed free of alcohol, washed three times and usually dissolved in Robinson's buffer devoid of glucose and Mg^{++} ions. The DNA content of the solution was determined both optically at 260 mμ and by the diphenylamine reaction

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(Dische and Shettles, 1951). None of the preparations used contained measurable quantities of ribose by the orcinol test (Mejbaum, 1939). However, there was wide variation in the protein content as determined by the Lowry procedure (Lowry et al., 1951). In some of the experiments, Difco trypsin or pronase was used to remove as much of the protein as possible. Fractionation of the DNA roughly according to molecular weight was accomplished by the DEAE cellulose paper pulp procedure of Ledoux (1965).

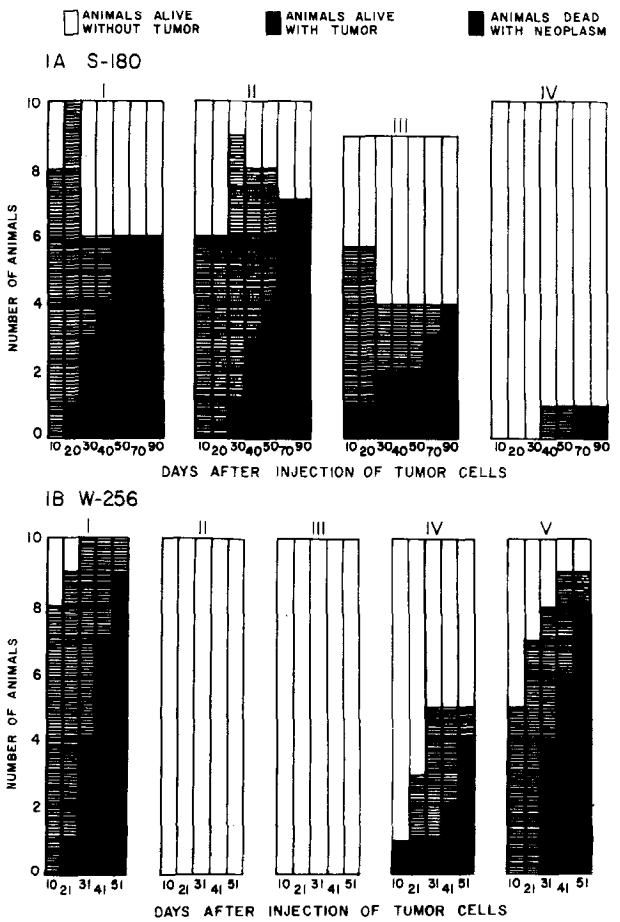

Figure I. The effect of DNA preparations on: (A) transplantability of sarcoma-180. Each animal was injected with 9.5 x 10^5 S-180 cells previously incubated in Robinson's buffer at 38°C for 60 min. with the following additions: group I, none; group II, 83 µg Difco trypsin; group III, deoxynucleoprotein containing 106 µg DNA; group IV, same as group III plus 83 µg Difco trypsin.

(B) transplantability of Walker - 256 carcinosarcoma. Each animal was injected with 9 x 10^5 cells incubated in Robinson's buffer for 60 minutes at 38°C, with the following additions: group I, none; group II, normal rat liver DNA, 140 µg/106 cells; group III, fresh W - 256 deoxy-

nucleoprotein, 158 μg DNA/10^6 cells; group IV, same as group III except DNA held 24 hr. at 3°C in Robinson's buffer; group V, same as group IV except DNA held 48 hr. at 3°C in Robinson's buffer.

Our experiments were performed by incubating the tumor cell suspensions, at 38° for 60 min. in Robinson's solution with and without (control) the different DNA preparations. After thorough mixing, the cell suspensions were injected subcutaneously without leakage in the flank of anesthetized animals. Subsequently the animals were examined by inspection and palpation at least every third day. Early tumors whether inflammatory or neoplastic were recorded but not distinguished. Only verified deaths from neoplasms were included in the cumulative totals indicated in the figures.

In the initial experiments, although only a few animals were involved the results were striking. Six mice were implanted with small pieces of S-180 tumor. Within 7 days all developed malignant growths and subsequently died of sarcoma. A second group of six mice were implanted with similar pieces of tumor together with roughly equal amounts of a deoxynucleoprotein derived from S-180. Within 7 days inflammatory nodules were palpable in all six mice but not one malignancy developed and to date, nearly one year later, all the animals are alive.

After several repetitions of this experiment, to better quantitate our results we followed transplantation with tumor cell suspensions. In a typical experiment, the results of which are shown in Fig. 1A, ten mice making up the control group I, were injected with 9.5 x 10^5 S-180 cells. The group II mice were injected with the same number of neoplastic cells previously incubated with trypsin (88 μg/10^6 cells). The S-180 cells injected into group III animals were incubated with S-180 deoxynucleoprotein (106 μg DNA/10^6 cells) and in group IV the animals were injected with S-180 cells which had been incubated with trypsin treated S-180 deoxynucleoprotein (106 μg DNA/10^6 Cells). While it is clear that trypsin alone does not suppress transplantability, exposure of the S-180 cells to S-180 deoxynucleoprotein causes a moderate drop in the number of animals developing neoplasms. However, when the deoxynucleoprotein preparation is treated with trypsin it produces a profound inhibition of S-180 transplantability.

In a typical example from among several subsequent experiments using rats implanted with W-256 carcinosarcoma cells (Fig. 1B) it is seen that deoxynucleoprotein (Group II, 140 μg DNA/10^6 cells) obtained from the livers of healthy rats is as completely effective an inhibitor of trans-

plantation as the deoxynucleoprotein derived from W-256 (Group III, 158 μg DNA/10^6 cells). Allowing the tumor deoxynucleoprotein to remain at 3°C in Robinson's buffer 24 hours (Group IV) or 48 hours (Group V) before incubating with the W-256 cells, produced progressive loss of the inhibitory capacity.

DNA fractions from DEAE paper pulp	S-180 takes[a]	S-180 DNA μg/10^6 cells	W-256 takes	W-256 DNA μg/10^6 cells
Fraction 3	0	57	0	137
"plus DNA'ase[b]	5	57[b]	7	137
Fraction 4	0	94	2	80
" plus DNA'ase	7	94	5	80
None	7	0	10	0

Table 1. The effect of deoxyribonuclease on the inhibition of tumor transplantability by various DNA fractions. The experimental procedure followed was exactly as outlined in Figure I. Both the W-256 and S-180 DNA preparations were digested with pronase (1 mg/ml) pH 7.4, 38°C for 60 minutes. Each was fractionated on DEAE paper pulp and the fraction numbers above refer to the successive fractions obtained by the Ledoux (1965) procedure. All DNA fraction were exhaustively dialyzed against Robinson's buffer devoid of glucose and divalent metals before incubation with tumor cells. Most fractions contained less protein than DNA and fraction 4(W-256) was devoid of measurable protein.

a) Animals with growing tumors are considered positive takes at 24 days. Each group contained 10 animals.

b) Treatment with DNA'ase I was at 38°C for 16 hours (0.5 mg/ml pancreatic DNA'ase).

These results clearly show an inhibition of tumor transplantability by treatment with various deoxynucleoprotein preparations but do not establish DNA as the inhibitor. Therefore we fractionated W-256 and S-180 deoxynucleoprotein preparations previously treated with pronase (Calbiochem) by the DEAE paper pulp procedure. Using Ledoux's (1965) convention, we have collected the following DNA fractions: 1 and 2 (mono - and small oligonucleotides); 3 (medium to low molecular weight) and 4 (intermediate molecular weight). Analysis of our fractions revealed that their protein content was very low and below the level of detection in fraction 4. As is shown in Table I the medium (fraction 3) and intermediate (fraction 4) molecular weight fractions are extremely inhibitory to tumor transplantability. Most significant is the fact that treatment of fractions 3 and 4 with deoxyribonuclease largely abolished the inhibitory effects of the

material. We interpret these results to mean that the inhbitory effect on transplantability is closely associated with DNA and that the DNA need only be of intermediate molecular weight to be effective.

Nucleic acids have been used previously to inhibit the growth of certain transplantable viral tumor cells. There are, however, basic differences between the previous experiments and those reported here. Niu (1965) has demonstrated that relatively high concentrations of homologous liver RNA will inhibit the growth of Nelson's Ascites tumor cells. However, RNA extracted from these tumor cells was without effect. Glick and Goldberg (1965) have also reported the inhibition of growth of mouse leukemia cells by exposure to homologous thymus DNA. In their case the subsutaneous injection of a cell suspension incubated with thymus DNA resulted in a 19% reduction in mortality rate. Again leukemia cell DNA failed to produce inhibition.

Our experiments have repeatedly shown inhibition of tumor transplantability by exposure of neoplastic cell suspensions to a DNA preparation extracted from two non viral malignant tumors of both rats (W-256) and mice (S-180), as well as from the liver of healthy rats. DNA prepared from calf thymus, salmon sperm and bacterial sources caused no inhibition in our system. Further work directed at the mechanism of this effect is now in progress.

Summary: DNA prepared from W-256 carcinosarcoma or Crocker sarcoma-180 when incubated with cell suspensions of these tumors markedly inhibited their transplantability. DNA derived from the liver of healthy rats was also inhibitory to transplantation of W-256 carcinosarcoma in rats. Treatment of deoxynucleoprotein preparations from these tumors with proteolytic enzymes enhanced their effectiveness as inhibitors while exposure of the DNA to deoxyribonuclease suppressed their inhibitory effect.

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