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Further Studies on a Transmissible Entity in Relation to the Control of Cancer in Mice

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Tumor-bearing mice of the C_3H/ST inbreds were used in this investigation. Of the 598 mice, 290 were controls, 248 received injections of 5-methyl cytidine dissolved in physiological salt solution, and 60 received injections of three nucleosides – adenosine, 6-methyl adenosine, and 5-methyl cytidine – also dissolved in physiological salt solution. The mice were divided into 6 groups based upon the number of generations of descent from a mouse injected with an alcohol-soluble liver extract. The criterion of tumor growth is the average increment of growth at the 20th observation period in the 7th week (3 per week). There is a cumulative inhibition of tumor growth, compared with controls, when 5-methyl cytidine is added to the effect of a transmissible entity which occurs in the lineal descendants of a tumor-bearing mouse injected with an alcohol-soluble liver extract and when the combination of three nucleosides in the same ratio of molar concentrations as in the liver extract is added to the effect of the transmissible entity.

KEY WORDS: transmissible entity, cancer in mice

Following the injection of an alcoholic extract of minced liver from several species, an entity (Strong, 1968) obviously involved in inhibiting the growth of spontaneous tumors of mammary-gland origin in mice appeared in the experimental mice (C_3H/ST). It was soon demonstrated that this entity was transmissible (Strong and Matsunaga, 1970). Evidence was obtained that the transmissible entity (TE) increased in potency in inhibiting the growth of cancer in mice between the first to about the sixth generation of lineal descent from a liver-extract-treated mouse. Following this lineage, the TE seemed to stabilize in intensity for a few generations, and then its ability to suppress cancerous growth in mice was gradually lost; that is, the growth capacity of a tumor in the 16th generation had returned to the growth rate of a tumor that had been determined in the O class, designated as the line of the original C_3H/ST inbreds before any liver-extract injection (Strong and Matsunaga, 1974).

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Analysis of these data on cancer control in relation to the TE suggests possible programs of research. Among the many suggestions, two appear to be most desirable: (1) to prove whether the TE is genetic or nongenetic and (2) to try to affect or alter the biological nature of the TE by the administration of one or more carefully selected elements derived from the alcohol extract of liver.

For the time being, the first approach to whether the TE is genetic or nongenetic has at least one hindrance. The presence of the TE was detected by ascertaining variations of the growth capacity of spontaneous tumors. But heterosis, which is maximal following an outcross, also affects the growth rate of spontaneous tumors. Consequently, the manner of hybridization required to detect a genetic TE would complicate a mendelian approach to an analysis unless sophisticated computer analysis, currently unavailable, were used.

Therefore the second method – trying to affect or alter the activity of the TE and its influence on the growth and fate of a spontaneous tumor – has been investigated.

The procedure of analysis became evident from the following facts that have been established: (1) Three nucleosides – adenosine, 6-methyl adenosine, and 5-methyl cytidine – and traces of several other entities were indicated in the alcohol extract of liver (Mittelman, personal communication).

(2) All three nucleosides were shown to be inhibitors of tumors in mice when used in the same molar concentrations as in the liver extract (Strong and Matsunaga, 1970). Higher or lower molar concentrations produced differences of potency in inhibiting cancer growth.

(3) The sum total of inhibition of tumor growth by the three nucleosides used one at a time was the same as that obtained by the alcohol-soluble liver extract (Strong and Matsunaga, 1973), and

(4) The TE had appeared in mice following the intraperitoneal injection of the liver extract (Strong and Matsunaga, 1970).

The obvious conclusion from these data was that each nucleoside should be measured in relation to the known variability of the TE entity to inhibit cancerous growth over a period of several generations of lineal descent.

The purposes of the present paper are (1) to report the effect upon the growth of cancer of injecting solutions of 5-methyl cytidine in the same lineal generations of descent where the TE had been measured and (2) to test a solution of the three nucleosides combined in the same molar concentrations as in the original liver extract. Generations 1 to 16 of lineal descent from an inbred mouse that had been injected with the liver extract were therefore used. The term F to indicate generations of descent is not used here since F is counted from descent following an outcross and in this experiment there has been no outcross – simply the continuation of the original C_3H/ST inbreds after more than 140 generations of lineal descent. Five-methyl cytidine was used first since it has been demonstrated to be the most powerful of the three nucleosides in inhibiting cancerous growth (Strong and Matsunaga, 1972).

MATERIALS AND METHODS

As in all previous reports dealing with tumor control with liver extracts and the nucleosides therein, inbred C_3H/ST and derived mutant C_3HB/ST mice have been used

exclusively. The present descent has now reached 165 generations of selected inbreeding without an outcross. The mice have been mated at weaning time. Subsequent offspring were raised to weaning age and mating continued to the appearance of a spontaneous tumor of mammary-gland origin. The tumor occurs in 95% of all mice which are used for breeding and appears between 5–25 months of age. However, for analysis only tumors between 5–17 months of age are used. The tumors are measured by calipers for the two longest diameters and these values are multiplied to give a measure of size.

The dose of the material to be tested is then determined and introduced by a subcutaneous injection. The dose at first is standard but it is somewhat reduced depending upon the physical appearance of the mouse. In case of a complete regression of the tumor, injections are stopped or used on the fifth or a later period of observation.

Since 5-methyl cytidine occurs as a .02 molar concentration in the original liver extract, this concentration, dissolved in physiological salt solution, was used in the first part of this experiment. In all, 5 series of tumor-bearing mice were used: series 6 received 1.54238 mg/inj; 7, 1.0285 mg/inj; 8, 0.51413 mg/inj; 9, 0.25706 mg/inj; and 10, 0.12853 mg/inj. (The evidence obtained is that within limits there is an inverse ratio between the amount of the 5-methyl cytidine dosage and the amount of tumor inhibition. Since the data on dose response have no bearing upon the analysis of the TE, they are not dicussed further in this report.)

The second part of this investigation was concerned with the effect upon tumor growth of a preparation of the three nucleosides, dissolved in physiological salt solution, in the same ratio as in the liver extract: .05 molar adenosine, 08 molar 6-methyl adenosine, and .02 molar 5-methyl cytidine. In this experiment, the 5:8:2 molar concentrations of the liver extract were divided by four, giving the formula 1.25:2:0.5. The series is labeled 1.252.5.

The mice were divided into groups depending upon the number of generations descended from the mouse receiving an injection of liver extract (the inducing material of the TE?): (1) 0, (2) E01–02, (3) 03–06, (4) 07–010, (5) 011–014, and (6) 015–016, Group 0 had no treatment with liver extract. They represent original C_3H/ST mice after 140 generations of controlled inbreeding. The other 5 groups are the offspring of female mice receiving the liver extract. 01 indicates one generation removed from the liver treatment (that is, the granddaughters); 02, two generations; and so forth. A few 015–016 mice have been produced but the numbers so far have been too few to be considered complete for this present analysis. (They are, however, presented in Fig. 1 and Table I).

RESULTS

Table I gives the number of tumor-bearing mice in each group. Classification of the six groups was based upon the number of generations the tumor-bearing mice were removed from an individual receiving the liver extract. Listed are the five series that were injected with the 5-methyl cytidine, combined series receiving 5-methyl cytidine, controls, and one series (1.252.5) in which three nucleosides – adenosine, 6-methyl adenosine, and 5-methyl cytidine – were present in the ratio 1.25:2:0.5. This ratio is 1/4 of the 5:8:2 molar concentration of each nucleoside in the original liver extract.

TABLE I. Number of Tumor-bearing Mice Treated, by Dosage and Generation

Series of mice	No. of animals	Dose of 5-methyl cytidine (mg/injection)	Mice untreated with liver extract	Mice descended from liver-extract-treated ancestor				
				1–2 generations after treatment	3–6 generations	7–10 generations	11–14 generations	15–16 generations
6	20	1.54238	3	5	1	9	2	0
7	48	1.02825	7	9	4	19	9	0
8	60	.51413	0	10	3	30	15	2
9	60	.25706	3	6	6	26	16	3
10	60	.12853	7	4	7	16	26	0
6–10 combined	248	.69407 (average)	20	34	21	100	68	5
Controls	290	–	24	77	36	68	53	32
1.252.5*	60	–	10	5	6	10	28	1

*This group received adenosine, 6-methyl adenosine, and 5-methyl cytidine in the ratio 1.25:2:0.5. In the original alcohol-soluble liver extract the molar concentration is adenosine, .05 molar; 6-methyl adenosine, .08 molar; and 5-methyl cytidine, .02 molar; or 5:8:2 (four times the 1.25:2:0.5 ratio).

In Fig. 1 the groups of tumor-bearing mice, classified by the number of generations removed from a liver-extract-treated ancestor, are on the horizontal line and the average increments of tumor growth at the 20th period of observation are on the vertical. The combined series receiving the 5-methyl cytidine are represented by the long-dash line, controls by the solid line and the three-nucleoside series by the short-dash lines.

The doses of 5-methyl cytidine, expressed as mg/kilo of body weight, for the five series of tumor-bearing mice receiving that nucleoside are as follows; for 6, 43.1 mg/kilo; 7, 28.8 mg/kilo; 8, 14.4 mg/kilo; 9, 7.2 mg/kilo; and 10, 3.6 mg/kilo. These values are based upon the average weight of 35.8 grams obtained for mice with their tumors.

DISCUSSION

Present data indicate a variable measurement of spontaneous tumor growth in mice for the different groups. These groups were classified according to the number of generations that the mice were removed, in lineal descent, from a mouse receiving a subcutaneous injection of an alcohol-soluble liver extract. The variability seems to be most pronounced in the controls, somewhat less in mice receiving 5-methyl cytidine, and least pronounced in the mice receiving a combination of the three nucleosides – adenosine, 6-methyl adenosine, and 5-methyl cytidine.

There is a similarity between the three curves beyond the 0 group but a greater divergence from the controls in the case of the three-nucleoside curve compared to the curve composing the data on the 5-methyl cytidine. In all cases, there is greater inhibition of tumor growth with either 5-methyl cytidine alone or the three nucleosides combined than in the controls.

The most obvious difference between the upper curve and the lower curves is the presence of either 5-methyl cytidine or combined nucleosides in the lower curves.

The greatest difference in the growth capacity of a tumor appears to be in mice of the 0 group, a phenomenon which had been observed by (1) the injection of the alcohol-soluble liver extract and (2) neomycin administered in the drinking water (Strong, 1968; Strong, 1970).

It is, therefore, suggestive that the effect of two inhibitors of cancer growth – a TE and either 5-methyl cytidine alone or in combination with adenosine and 6-methyl adenosine – is cumulative over a period of sixteen generations of a lineal descent.

In cancer chemotherapy, a cumulative effect on inhibition is frequently obtained when two agents affect two distinct mechanisms of action. It is well to keep these observations in mind in attempting to understand the role of each moiety on cancer control in mice.

It is also important to consider that 5-methyl cytidine or a combination of nucleosides, plus the TE, may be more than casually related to each other, based upon their actual origin.

Since the TE was induced (?) or at least disclosed by an injection of an alcohol-soluble liver extract, a mixture of several components, the present experiment is not ideally designed to fully analyze the cumulative effect on tumor growth by TE and either 5-methyl cytidine or the combination of three nucleosides. This discrepancy was predicted some time ago and it was hoped that part of the confusion might be resolved by using

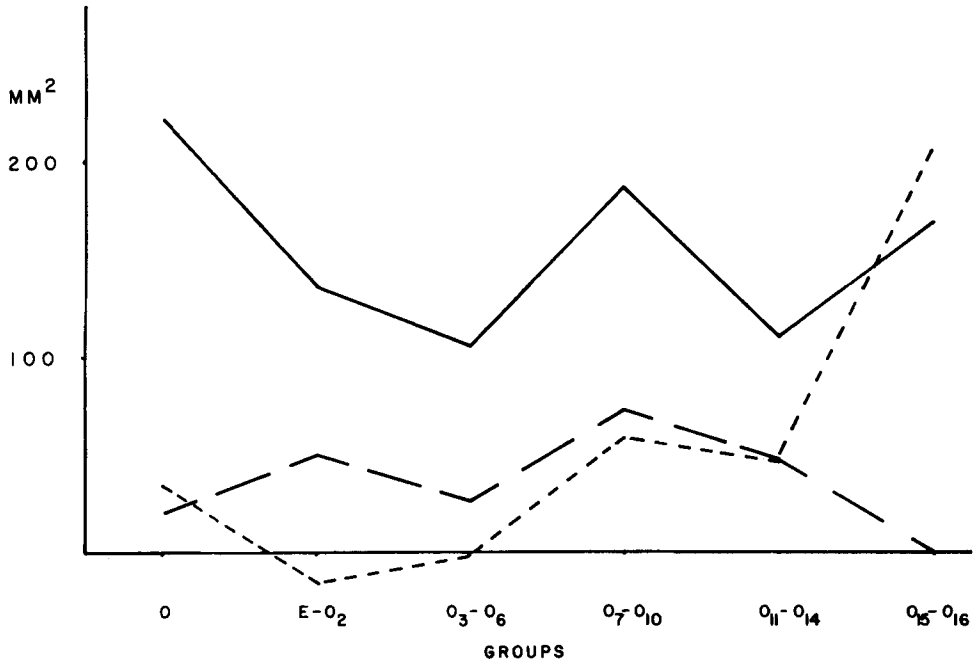

Fig. 1. The vertical line measures increment of tumor growth in mm^2. The 6 groups on the horizontal line include: 0, not descended from liver-extract-treated mice; $E\text{-}0_2$, 1st–2nd generations of lineal descent from liver-extract treated mice; 0_3-0_6, 3rd–6th generations; 0_7-0_{10}, 7th–10 generations; $0_{11}-0_{14}$, 11th–14th generations; and $0_{15}-0_{16}$, 15th and 16th generations. Mice receiving 5-methyl cytidine are represented by the long-dash line, controls by the solid line, and mice receiving three nucleosides, series 1.252.5, by the short-dash line.

mice with a TE that appeared following the injection of 5-methyl cytidine exclusively. These mice are now developing spontaneous tumors, but it will take considerable time to analyze the data.

A similar experiment, dealing with a possible different TE induced (?) with an injection of the three-nucleoside combination, should also be tried.

As in previous observations and discussions on the use of liver extracts and nucleosides on cancer control in mice, the reasons for an observation should be eventually resolved – but even more important is that cancer control is already being obtained.

The new data indicate a cumulative effect of tumor inhibition (with TE and either 5-methyl cytidine or a combination of three nucleosides) very close to the base line of tumor growth, the appearance of averaged negative values of increments which signify the regression of tumors, and in some cases, complete cancer control.

CONCLUSIONS

There is cumulative effect on the inhibition of cancer growth in mice with a transmissible entity and either 5-methyl cytidine or a combination of three nucleosides – adenosine, 6-methyl adenosine, and 5-methyl cytidine.

This cumulative effect has been demonstrated over a period of 16 generations of lineal descent from a mouse in which the transmissible entity was detected.

The maximal effect of either 5-methyl cytidine or the combined three nucleosides on suppressing cancer growth is obtained in the group not descended from mice in which the transmissible entity was detected.

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