Journal of Surgical Oncology 9:99–103 (1977)

Increased Effect of a Transmissible Entity on the Control of Cancer in C_3 H/St Mice

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Three hundred and seventy-one mice of the C_3H/St inbreds bearing spontaneous tumors of mammary gland origin have been used in this experiment. All mice studied were lineal descendents of a tumor-bearing mouse which had been injected with a liver extract. In this descent there appears to be a transmissible entity presumably induced by the injection of the liver extract.

Following 15 generations of inbreeding after the appearance of a transmissible entity, a second sudden reversal of effect on cancer growth in mice has been indicated.

This change has progressively altered the growth of cancer from a very low effect to a maximal effect, i.e., to a complete regression of a high percentage of the tumor.

The maximal effect of the transmissible entity is the complete suppression of the growth of cancer during, at least, through the 25th period of observation, thus producing negative values of tumor growth.

All these effects of the growth and fate of spontaneous tumors of mammary gland origin in mice have been obtained in a single lineal descent of C_3H/St inbreds without resorting to any outcross.

Key words: transmissible entity, cancer

INTRODUCTION

In 1970, data were presented that indicated the presence of a "transmissible entity" for the partial control of a spontaneous tumor of mammary gland origin in mice of the C_3H/St inbreds. At that time, the entity had been traced through 9 generations of lineal descent, following the subcutaneous injection of a specially prepared tumor-inhibiting liver extract. It was found that the entity increased in potency in controlling cancer in mice of succeeding generations. Following this report, 4 additional papers (2,3,4,5) have been published, dealing with newly acquired data on the characteristics of this entity. In the latest paper (5), it was concluded that "there is a cumulative inhibition of tumor growth, compared with the controls, when 5 methyl cytidine was added to the effect of the 'transmissible entity' which was shown to be present in the lineal descendents of a tumor-bearing mouse which had been injected with an alcohol-soluble liver extract." This cumulative effect, obtained when the combination of 1 of the 3 nucleosides was used in

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the same molar concentration as had been detected (by Mittelman) in the liver extract, emphasizes the possibility of entertaining some correlation between a host-derived inhibitory entity and the probable inciting components of an inducing mechanism derived from a liver extract.

The purpose of the present publication is to report on the growth of spontaneous tumors obtained in mice through 22 generations of lineal descent following the injection of the liver extract into a parent with a spontaneous tumor at that number of generations removed from the same descent. The data reported now for the $O_{16}-O_{22}$ generations of descent are new, but are added to the data for O_1-O_{15} which have been reported previously (5).

MATERIALS AND METHODS

As was the case in former papers dealing with tumor control with liver extracts and the 3 nucleosides (adenosine, 6 methyl adenosine and 5 methyl cytidine) contained therein, the inbred C_3H/St and derived mutant C_3HB/St mice were used in this experiment. Two or 3 females were placed with 1 male (always a brother) at weaning age. These mice were kept together for breeding purposes until the appearance of a spontaneous tumor in a female or the death of either of the potential parents. A tumor-bearing mouse was separated from the male and placed in an isolated cage until death.

In this experiment, there were no experimental proceedings of injections or other treatments for the tumor-bearing mice. These mice, however, should not be considered as controls, since a liver extract known to affect the growth and fate of spontaneous tumors has been injected into 1 or more of the cancer proband's ancestors of 1–22 generations previously.

The tumors were measured in the 2 longest diameters by verniers 3 times weekly. The 2 values were multiplied together as a measure of size of the tumors. This original value was deducted from the size of the primary tumor in order to estimate the increment of growth. In this experiment, the average increment of growth at the 25th period of observation is compared for the different classes. In this case, the classes were determined by the number of generations distant from the original mouse bearing a spontaneous tumor that had received the liver extract. It has been suggested that the injection of the liver extract has had an effect not only upon the growth and fate of the spontaneous tumor, but also upon an "induction effect" upon the appearance of a "transmissible entity" now under consideration. These generations of descent were obtained by continued inbreeding (brother to sister matings only) without an outcross. Therefore, the number of generations of descent are referred to as O_1-O_{22} rather than F_1-F_{22} which would imply an outcross.

RESULTS

The data on a measure of tumor growth, and on average increments of growth at the 25th period of observation, are presented in Table I and Fig. 1.

Table I gives the number of tumor-bearing mice in each of 7 composite classes from 1 lineal descent. None of these derived descendents of a mouse which had received an injection of a liver extract has ever received any treatment of their mammary gland-derived tumor.

Figure 1 presents the data on analysis of growth of spontaneous tumors for mice of the 7 combined classes of descent from a single lineal descent without an outcross. The

TABLE I. Number of Tumor-Bearing Mice in 7 Composite Classes From 1 Serial Descent (No Treatment)

Class	Generations	No. mice
1	0	24
2	E 01 02	77
3	03 04 05 06	36
4	07 08 09 010	68
5	011 012 013 014	48
6	015 016 017 018	61
7	019 020 021 022	57
Total		371

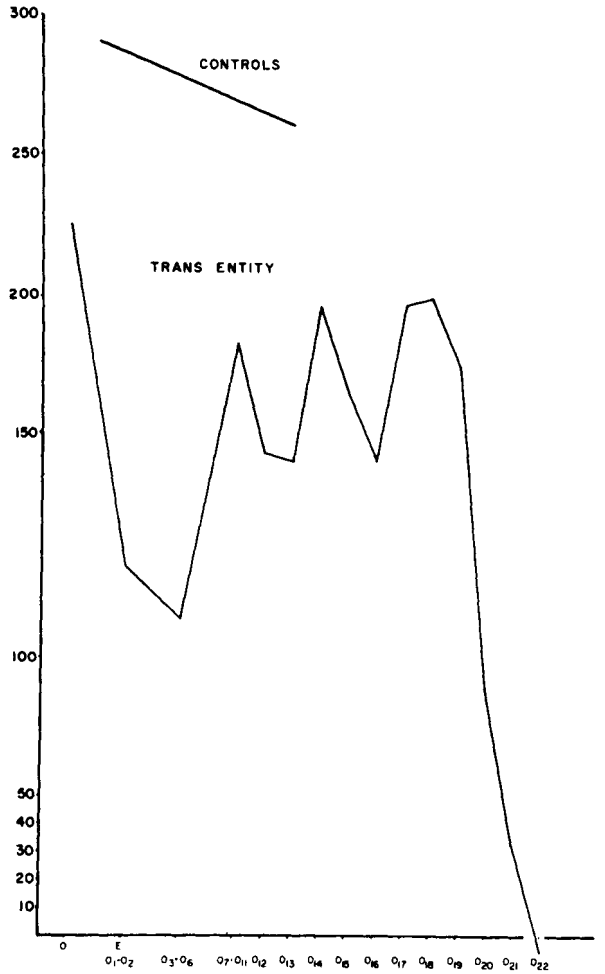

Fig. 1. Data on the average increments of tumor growth at the 25th period of observation. Classes 1–4 and subsequent generations $O_{11}-O_{22}$ are spaced along the base line, while average increments of growth expressed in mm^2 are given on the vertical line.

mice of successive generations of the 7 combined classes bearing cancer are spaced on the base line. The average increments of growth at the 25th period of observation (3 per week) are presented on the vertical line.

Data for classes 1–6 (mice O_1–O_{15}) were given in a previous publication. New data of class 6 (completed to O_{18}) and series 7 (mice O_{16}–O_{22}) are now reported, but shown in proper sequence of descent to the previously published data (O_6–O_{15}) (4).

DISCUSSION

Observations have been reported on the growth of spontaneous tumors occurring in mice for 22 generations of a single lineal descent derived from a mouse which had received a subcutaneous injection of a liver extract. It is known that 3 nucleosides were present in the original liver extract (Mittelman). These are 1) adenosine 0.05 molar concentration, 2) 6 methyl adenosine 0.08 molar concentration, and 3) 5 methyl cytidine 0.02 molar concentration. No outcross of a mouse of the C_3H/St inbreds has ever been made, so that the variations obtained through continued inbreeding after 165 pedigreed generations must be due to some other mechanism than genetic recombinations of existing genes.

The data indicate quite convincingly that something in the mechanism of the growth of a spontaneous tumor in mice is changing. It is obvious that this change takes place in both directions of variations, as follows: Following the appearance of a sudden change in the potency of some mechanism of suppressing the growth of cancer through 6 generations of descent, there occurred a period of fluctuations of effect of suppressing cancers and then a stage of exerting practically no effect on cancer growth. Then it appeared that the transmissible entity had become ineffective in influencing cancer. Hence the conclusion derived after observations on 15 generations of continuing inbreeding – even after 165 generations of restricted brother-to-sister mating – was that "by 15 generations of lineal descent, the transmissible entity appears to be either lost or, at least, inactivated in suppressing cancer" (4).

Beginning with the 16th generation, however, there has been an ever-increasing effect upon the suppression of growth of cancer. This second sudden change in a biological or biochemical characteristic of suppressing cancer (first sudden change between O_1–O_6 and now the sudden change in O_{16}–O_{22}) has continued for another 6 generations of a single lineal descent. Thus, in O_{22} all mice with spontaneous tumors (8) have regressed their tumors, thus giving a negative value of –3 for the measurement of increment of tumor growth at the 25th period of observation. However, a few of these tumors from O_{22} mice have shown a recurrent positive slow growth after the 25th period.

It seems most likely that the "transmissible entity" may be an unstable biological or biochemical entity within the body of the host-bearing cancer, and that it is sensitive to sudden changes in potency in affecting the growth rate of a spontaneous tumor as measured by increments of growth. While not in a period of sudden change, the entity appears to fluctuate in potency to control cancer in mice of succeeding generations of descent.

CONCLUSIONS

To the conclusions derived from the study of a transmissible entity in relation to the control of the growth of spontaneous tumors of mammary gland origin in mice may now be added the following, some of which are partly revised from conclusions based on previously obtained data (1,2,3,4,5).

1. Following 15 generations of inbreeding after the appearance of a transmissible entity, a second sudden reversal of effect on cancer growth in mice has been indicated.

2. This change has progressively altered the growth of cancer from a very low effect to a maximal effect, i.e., a complete regression of the tumor.

3. The maximal effect of the transmissible entity is the complete suppression of the growth of cancer during, at least, through the 25th period of observation, thus producing negative values of tumor growth.

4. All these effects of the growth and fate of spontaneous tumors of mammary gland origin in mice have been obtained in a single lineal descent of C_3H/St inbreds without resorting to any outcross.

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