Growth of an Inbred Stock Mammary Adenocarcinoma after Transplantation at Different Times Following Prior Inoculation*

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The incidence of lung metastases produced by transplanted mouse tumors has been found to be proportional to the size of the primary growth (4, 5). Since metastases are essentially the result of an autotransplantation of tumor cells detached from the primary growth, it was tentatively thought that perhaps the time at which the metastatic spread occurs might be coincident with an increased susceptibility of the host toward a reinoculation of the same tumor. If this were the case, animals growing a transplanted tumor might show a progressive increase in susceptibility to a second transplant made at different time intervals after the first inoculation.

METHODS AND MATERIALS

To test this hypothesis, experiments were carried out in the following way: Young mice of both sexes of the ZBC1 stock were used as recipient hosts. ZBC mice are back-cross hybrids able to grow tumors arising in mice of the Z(C3H) strain. The tumor used was a mammary adenocarcinoma which arose spontaneously in a Z(C3H) breeder mouse and has been maintained in this laboratory for 53 successive passages into more than 2000 mice of their own strain (F1 hybrids and ZBC hybrids), growing progressively and killing the hosts in all instances. Furthermore, this tumor has maintained its original genetic characteristics, since under normal conditions it does not grow in any strain of mice other than Z(C3H), F1 hybrids, and ZBC hybrids.

Two types of experiments were performed. In one, the tumor was transplanted into the subcutaneous tissue of the tail. This was accomplished by preparing a tumor homogenate suspended in saline as an 8 per cent concentration and injecting 0.05 cc. of the suspension, per mouse, approximately 2 cm. from the tip of the tail. The injection was made with a 27-gauge needle attached to a tuberculin syringe, special care being taken to avoid the introduction of the needle into the tail veins.

At 12, 17, and 22 days after the caudal transplant, tails of certain groups of mice (with caudal tumors having a mean volume of 0.12, 0.3, and 1.03 cc., respectively) were amputated by being clipped off near the root with a pair of hot-sterilized scissors. No anesthesia was used. This method proved to be excellent, since the entire operation could be done rapidly and without appreciable bleeding. The removal of the tumor growing in the tail was immediately followed by the subcutaneous inoculation of 0.25 cc. of a 5 per cent concentration of a second tumor cell suspension into the right groin.

The tumor used for the second inoculum was taken from the same group of donors used for the caudal transplant. For each experimental group a control group of mice was given injections in the groin of the second tumor suspension following amputation of their normal tails. After the second implant, mice were inspected once every other day for the appearance of tumors of no less than 8 mm. in diameter at the site of implantation, and the progressive incidence of tumors in the various groups was determined.

In another set of experiments the tumor was first implanted into the subcutaneous tissue of the right ear by the making of a small incision in the skin at the base of the ear and the introduction of a bit of tumor tissue between the skin and the ear cartilage, with the aid of a pair of fine-pointed forceps. Thirty days after the transplant, the ear carrying the tumor was clipped off, and a second tumor inoculation was made into the right groin.

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as in the first experiment. Control mice received only the second transplant after their normal ears were clipped off, and the appearance of tumors resulting from the groin inoculation was determined in both groups.

In nearly all mice (97 per cent) first implanted either in the tail or in the ear, the tumor grew successfully, and those few animals in which the primary tumor failed to grow were not used in the experiments.

RESULTS

Prior tail inoculation.—The results of these experiments are summarized in Chart 1. As will be seen in the chart, the incidence of tumors following a secondary implantation, in the group of 70 inhibition of tumor takes. Of the 48 experimental animals previously inoculated in the tail, only 4 per cent developed tumors, whereas in 95 per cent of the 51 control animals the inoculation in the subcutaneous tissue of the groin was followed by the development of tumors.

It will be noted that, of a total of 171 control mice inoculated, only twelve (7 per cent) failed to develop tumors. Even these few failures might be explained on the basis of imperfect inoculation technic, since in a few instances leakage of tumor suspension was noted which might have prevented the implantation of a sufficient number of tumor cells.

Prior ear inoculation.—To determine whether similar results would be obtained when the original animals reinoculated in the groin 12 days after the original implantation of tumor in the tail, was almost the same as that in the group of 68 control animals (94 and 96 per cent, respectively). However, as may be seen in the chart, there was a significant difference in the time of appearance of tumors in the two groups. Those previously inoculated in the tail developed tumors more rapidly than did the controls.

In the group inoculated at 17 days after the original implantation of tumor in the tail, there appeared to be inhibition in the production of tumors, since almost half (48 per cent) of the 66 mice in the experimental group failed to develop subcutaneous tumors upon reinoculation of tumor suspension in the groin. In contradistinction, 90 per cent of the 57 control animals developed tumors. This difference is statistically significant at the 1 per cent level. In the group reinoculated in the groin 22 days after the original implantation of tumor in the tail, there was an almost complete tumors were growing at a site other than the tail, a similar experiment was performed in which the ear rather than the tail was the site of the original inoculation.

The results of this experiment are summarized in Chart 2. It is clear from this chart that, as with mice previously inoculated in the tail, those having a prior growth of tumor in the ear showed a significant inhibition of tumor takes following a second transplant. Only 35 per cent of the experimental group developed subcutaneous tumors, as compared with 100 per cent takes among the control animals.

It is interesting to point out that 33 per cent of the mice in the 17-day group and all animals of the 22-day group, as well as those implanted in the ear, had well developed metastases in their lungs produced by the primary growth, as demonstrated by post-mortem examination of their lungs under a dissecting scope. This was clinically evidenced in most cases by the presence of marked dyspnea,
which in some instances resulted in sudden, unexpected death of the host. However, there were no signs of malnutrition or cachexia due to the presence of metastases, since the determination of the mean body weights at the end of the experiment in the experimental as well as in the control groups did not reveal significant differences (20.01 ± 0.5 gm. for the experimental group and 19.53 ± 0.22 gm. for the controls).

**DISCUSSION**

The results of these experiments are most provocative. In the first place, except for one observation (at 12 days) they are at variance with the hypothesis which provoked the study. It was originally postulated that, since metastases represent in essence autotransplants of tumor cells detached from the primary growth, second subcutaneous transplants made during a period when metastases were taking place or were establishing themselves might take more rapidly or grow faster than in normal controls. Observations in animals in which reinoculation was made 12 days after the original implantation of tumor were in agreement with this prediction. In these mice, the incidence of tumors was as great as in the controls, and the time of appearance of tumors seemed to be significantly shorter than that of the controls, indicating what might be interpreted to represent an enhanced susceptibility to tumor growth. Re-transplantation of the tumor at 12 days was chosen as a critical interval, because previous studies (4) indicated that metastatic spread of the primary tumor might begin at this time.

The enhanced susceptibility to subcutaneous implantation occurring at the time metastases appear to be taking place, as demonstrated in this experiment, suggests that at this particular time the host may be particularly susceptible to autotransplantation. The mechanism involved in the enhanced susceptibility demonstrated at this time remains enigmatic and deserves more experimentation.

Somewhat surprising, then, was the observation that resistance to secondary tumor transplantation had developed 17 days after initial implantation, at a time when lung metastases had been clearly established in many of the animals. This was more clearly seen in the group reinoculated 22 days after the initial implantation in the tail as well as in the group reinoculated 30 days after ear inoculation.

The reason for the enhanced resistance to a second transplant under the circumstances is not clear. Since most animals resisting the second inoculation exhibited lung metastases which provoked a marked respiratory insufficiency, it might perhaps be possible to interpret the resistance to second inoculation as owing to some type of nonspecific circulatory disturbance which could prevent a proper vascularization of the graft. On the other hand, it might also be conceivable that the first tumor could induce some form of immunity. In fact, Andervont (1) by the use of a similar technic was able to show immunity in mice reinoculated with Sarcoma 180 when tested 2 weeks after the caudal transplant. Bittner (2) confirmed these results for Sarcoma 180 in some inbred stocks of mice but not in others. Similarly, using fibrosarcoma S621, originally induced in a mouse of the BALB/cJax subline by a single injection of methylcholanthrene, Fink et al. (3) found that immunity by prior caudal inoculation could be demonstrated in 66 per cent of mice of the BALB/cSn subline, in 15 per cent of mice of the BALB/cJax and in 6 per cent of the C57, BR/Sn X BALB/cSn hybrids. Therefore, it seems that whereas this tumor could induce immunity in one subline of mice (BALB/cSn), it was not able to do so in either its own original strain (BALB/cSn) or in BALB/cSn F1 hybrids.

Our results are in agreement with those reported earlier by one of us (2) with a similar inbred stock Z(C3H) mammary tumor transplanted into mice of their own strain, F1 and ZBC hybrids, since no immunity was found in any of these mice when the reinoculation was made 2 weeks after the caudal transplant. In fact, our data obtained with a similar test system indicate that at 12 days there was indeed an increased susceptibility rather than immunity. On the other hand, when reinoculation was made later, evidence of some type of
immunity was clearly obtained. This constitutes, to our knowledge, the first instance in which, by this technic, immunity could be demonstrated by the use of mammary tumors in inbred mice.

The nature of the immunity demonstrated in these experiments has not been clarified, since preliminary attempts to transfer this to normal mice with serum or cells of the resistant animals have been unsuccessful.

SUMMARY

Experiments with mice to test host susceptibility to the reinoculation of the same tumor made at different time intervals following either a tail or ear transplant have shown the following results: (a) If reinoculation was made 12 days after the caudal transplant, there was an acceleration in the appearance of new tumors in this group as compared with the control group, although the final incidence was almost the same in both groups. (b) If reinoculation was made at 17 days, a significantly lower incidence was obtained. (c) When the reinoculation was made 22 days following the tail transplant, almost complete inhibition was obtained. (d) When the reinoculation was made 30 days after a prior transplant in the ear, qualitatively the same results were obtained.

REFERENCES
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