Continuous Growth of Isotransplants of a Mammary Tumor Associated with the Development of Immunity in Mice*

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Recently, we have reported that mice growing an inbred mammary tumor either in the tail or in the ear showed varying degrees of susceptibility toward the subcutaneous reinoculation of the same tumor at various times after the first transplant (1). The second tumor grew faster in the group reinoculated 12 days after the first transplant than in the corresponding controls. However, those reimplemented at 17, 22, and 30 days showed a marked resistance. These later results were interpreted as owing to the development of some form of immunity. The fact that at the time of reinoculation the resistant animals had well-developed metastases in their lungs originating from the first growth brought up the question whether the presence of metastases at the time mice became immune was an interdependent phenomenon or whether both events, i.e., metastases and immunity to the subcutaneous transplant, were entirely independent from one another.

To investigate this point and as an extension of the previously reported results, an experiment was planned in such a way as to be able to test for immunity in mice carrying a prior transplanted tumor for as long as 30 days without producing lung metastases. Previous studies (2) indicated that 12 days after tumor inoculation into the subcutaneous tissue of the tail appeared to be the critical time at which the primary tumor begins to metastasize to the lungs. Therefore, in these experiments in order to prevent the metastatic spread of the tumor, it was decided to excise and reimplant tumor every 10 days to different locations in the same mouse until a 30-day period of continuous growth of tumor was completed. At that time and after a final removal of the tumor, mice were tested for susceptibility to a subcutaneous graft of the same tumor in the groin.

MATERIALS AND METHODS

Young male mice of the ZBC1 stock were used as recipient hosts. ZBC mice are back-cross hybrids able to grow tumors from mice of the Z(C3H) strain. The tumor was an adenocarcinoma of the mammary gland which arose spontaneously in a breeder mouse of the Z(C3H) stock and has been maintained in this laboratory for 55 successive passages into mice of the same strain, F1 hybrids, or ZBC hybrids, growing progressively and resulting in death of all recipient animals. Furthermore, this tumor has maintained its original genetic characteristics, since under normal conditions it does not grow in any strain of mice other than those listed above.

The experiment was conducted in the following way: Mice were given grafts of this tumor in the subcutaneous tissue of the left ear by means 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. Ten days after the transplant, mice were inspected for ear tumors, and only those showing unequivocal signs of tumor growth were submitted to amputation of the ear by clipping it off with scissors without anesthesia. This was followed by the implantation of another tumor of the same generation into the subcutaneous tissue of the right ear. After a period of another 10 days mice showing growth of this tumor were again submitted to amputation of the right ear, followed by a third transplant to the subcutaneous tissue of the tail. The tail inoculation was carried out by preparing an 8 per cent tumor homogenate in saline and injecting 0.05 cc. of this suspension into the subcutaneous tissue of the tail.

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the tail at approximately 2 cm. from the tip of the tail. Ten days after the caudal transplant all mice were submitted to amputation of their tails, immediately followed by the subcutaneous inoculation of a tumor of the same generation into the right groin. The tumor for the groin inoculation was prepared as a 5 per cent tumor cell suspension in saline, and 0.25 cc. was injected per mouse.

Two groups of mice were prepared as controls for the tumor transplant in the groin. One of these was composed of animals which had been carrying a previously transplanted tumor in the left ear for 30 days, and the other was composed of normal untreated mice.

After inoculation in the groin, the three groups of mice were inspected every other day for the appearance of subcutaneous tumors at the site of inoculation, and after a period of 45 days the animals were sacrificed, the lungs removed, and the presence of metastases over their surface was determined under a dissecting microscope (1.3 x 10 magnification).

RESULTS

The results obtained are shown in Table 1. In group I, of the 30 mice first transplanted with tumor into the left ear, twenty (or 67 per cent) had actively growing tumors when examined at 10 days after the inoculation. When these twenty animals were subjected to removal of the left ear, which bore the tumor, and were subsequently given inoculations of another tumor in the right ear, the number of mice showing new growths at this site after 10 days was nineteen (95 per cent). Finally, when the tails of these nineteen animals were amputated followed by the subcutaneous inoculation of tumor into the groin, the final incidence of tumors appearing at this site after 45 more days was 11 per cent. None of the mice in this latter group had macroscopic lung metastases when sacrificed at the end of the experiment.

On the other hand, of a total of twenty mice in which the first tumor had grown in the left ear only for 30 days (group II) and which were submitted to reinoculation of tumor into the groin after their left ear was clipped off, there was also an inhibition of tumor takes in the groin, since only four animals (20 per cent) developed tumors at this new site. However, as indicated in the table, eighteen of these mice (90 per cent) had metastatic tumors in their lungs from the continuous growth of the primary tumor in the left ear. Finally, of a total of twelve control mice (group III) receiving only the tumor inoculation in the groin, all developed subcutaneous tumors at this site.

DISCUSSION

The results of the experiments described are a confirmation and extension of similar work reported earlier (1). Specifically, it is confirmed that mice previously bearing a transplanted mammary tumor fully histocompatible with the hosts show varying degrees of susceptibility to a reinoculation of the same tumor made at various time intervals after the first transplant. This was clearly seen in mice given successive transplantations of tumor first in the left, then in the right, ear and finally in the tail. In fact, under these conditions, mice in which a tumor had grown in the left ear for 10 days showed an acceleration in the takes of a second transplant made in the right ear.
when these same animals received a third inoculation in the tail, the tumor grew successfully in only a few. The observed incidences of takes 10 days after each transplant were as follows: 67 per cent when the inoculation was made in the left ear, 95 per cent when in the right ear, and 11 per cent when in the tail.

On the other hand, the results obtained when the tumor was inoculated into the groin of mice bearing either a single tumor in the left ear or three successive transplants from ear to ear to tail for 30 days showed, as was expected from previous results, that most animals from both groups did not develop subcutaneous tumors, in contrast to takes in all instances in the group of normal controls. However, it is interesting to point out that mice receiving three tumor inoculations prior to the groin transplant did not exhibit metastases when sacrificed at the end of the experiment, whereas of those previously bearing a single tumor in the ear metastases were found in the lungs of 90 per cent. The absence of metastases in those animals previously inoculated with tumor on three successive occasions may be explained as owing to the fact that each tumor was allowed to grow for a limited time only (10 days) and subsequently removed before metastatic cells could detach from any one of them. On the other hand this was expected in view of previous findings (2). However, the fact that both groups of animals, with and without metastases, were similarly resistant to the tumor inoculation into the groin indicates that the presence or absence of metastases at the time the mice became immune does not play a role in the production of immunity by the previous growth of the tumor.

SUMMARY

It was confirmed that mice with either a single tumor transplant growing in the left ear for 30 days or with three successive inoculations of the same tumor, each being left at each location for only 10 days, were then resistant to a subcutaneous inoculation of tumor made into the groin. The fact that those animals receiving successive inoculations and excision of the developing tumors did not exhibit metastases in their lungs but did demonstrate subsequent resistance to a further grafting of the same tumor indicates that the lung metastases previously observed accompanying this type of immunity were in no way related to the presence of such immunity.

REFERENCES

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