The Production of Tumors by Transplantation of Normally Appearing Liver Cells from Animals Previously Injected with Methylcholanthrene*†

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Several years ago we observed that injected minced liver of C3H mice, previously given methylcholanthrene and bearing subcutaneous sarcomas of the groin, occasionally resulted in rapidly growing tumors at the site of injection. As such tumors usually developed within three weeks and resembled the primary methylcholanthrene growth (polymorphous cell fibrosarcoma), it seemed probable that living cells of a metastasis had been transferred.

Repeated histological studies of samples of liver used in transplantation, however, failed to reveal metastases in the liver. In fact, little or no abnormality was found in livers giving successful takes. Such tissues showed suspicious cells only occasionally. Variations in nuclear size, hyperplasia and mitoses (the mitoses being in liver cord cells rather than in secondary tumor cells) were observed. A few liver cells contained suggestive intranuclear inclusion bodies. Occasional multinucleated cells, possibly megakaryocytes, were seen not only in the liver sinusoids but also in the spleen. These cellular features, however, could be found in normal animals of the same age. The significant point is that although tumor metastases were not visible, either grossly or microscopically, rapidly growing tumors were occasionally found to develop at the site of injection of such seemingly normal liver tissue.

Stimulated by the recent publications of Steiner (4), Kleinenberg, Neufach, and Shabad (3), Des Ligneris (1), and Hieger (2), who reported the production of tumors in mice by the injection of lipoid extracts of human liver, we extended the original transplantation experiments. These are reported here.

Experiments

The material for this study was derived from: I. 62 C3H mice bearing sarcomas induced by methylcholanthrene, measuring 1 to 2 cm. in diameter; II. 21 C57 black mice bearing methylcholanthrene tumors of similar size; III. 30 C3H mice injected 7 weeks previously with methylcholanthrene but not yet possessing induced tumors; and IV. 37 normal C3H mice.

The freshly excised sterile liver of each host was cut into 6 or 8 pieces, several of which were selected at random for fixation in formalin for microscopic study. The remaining pieces were minced and to the hash an equal volume of saline was added. Approximately 0.2 cc. of this minced tissue was injected subcutaneously into the groin area of 5 to 10 recipient mice. In more recent experiments a concentrated cell-free filtrate,[1] obtained from thoroughly ground liver, was injected into 3 or 4 additional animals. In about half of the experiments in which tumor-bearing animals served as the source of the transplanted tissue, the spleen was also minced and injected into several animals.

Out of 62 groups of recipient mice injected with liver from C3H animals bearing methylcholanthrene tumors, tumors arose in one or more animals in 7 groups (or in 11 per cent of the groups). In 3 of the 7 groups giving positive results, all surviving recipient animals developed tumors; in 2 groups, 4 out of 5 surviving animals developed tumors; in the remaining 2 groups, approximately half of the animals developed tumors. With but one exception the tumors were detectable within 3 weeks; the exceptional one was

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[1] Approximately one third of the liver of the host was hashed and thoroughly ground in fine sand. Five cubic centimeters of Ringer's solution was added to the macerated tissue and the mixture stirred for 5 minutes, then allowed to stand an additional 10 minutes. The suspended cells and debris were removed by centrifugalization (2,500 r.p.m. for 20 min.). The recovered supernatant fluid (4-5 cc.) was drawn into a 5 or 10 cc. syringe and filtered through a ½ inch Seitz disc (B-D No. F D) used in a Swinney B-D filter adapter (No. 423 F A). The filtrate was passed through a second disc to insure complete removal of the cells. Approximately 0.5 cc. of the filtrate was then injected into each of three or four animals.

We were unable to detect cells in the filtrate after passage through the second disc.

not observed until after 6 weeks. Tumors developed in both males and females, and in young and old animals, in approximately equal numbers.

The livers of the host animals were all found grossly and microscopically to be free of metastases. The cells, as seen in histological sections, were apparently normal. The tumors arising at the sites of injected liver were polymorphous cell fibro-sarcomas similar microscopically to the induced tumors of the hosts.

In several instances of successful tumor growth following injection of liver from C3H mice into C3H recipients, growths were not obtained when the recipients were C57 black mice. Furthermore, the livers of C57 black mice bearing subcutaneous methylcholanthrene tumors failed to produce growths in either C57 or C3H recipients.

In no instance did tumors develop from the injection of cell-free filtrates, or from minced spleen. Neither did tumors develop at the site of injection of liver from a normal animal nor from a methylcholanthrene injected animal which had not yet developed an induced tumor.

Assuming the possibility of the transference of tumor cells, which admittedly is the most plausible explanation for the developing tumors despite repeated failure to find such liver metastases, the unusually rapid development of these tumors is a feature which warrants further study. The small amount of tumor tissue which might conceivably be injected appears to have produced results out of proportion to those which occurred when a mixture of 1 part of minced primary tumor and 9 parts minced normal liver tissue was injected under similar conditions.

Summary

Freshly excised liver tissue, apparently normal and free from metastases, from mice bearing tumors induced by injections of methylcholanthrene was injected subcutaneously into normal mice. Tumors arose at the site of injection in a number of animals. Cell-free filtrates of the same liver tissue, injected in the same manner, did not induce the formation of tumors.

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