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ARMED FORCES INSTITUTE OF PATHOLOGY

ATLAS OF TUMOR PATHOLOGY

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TRANSPLANTABLE AND TRANSMISSIBLE TUMORS OF ANIMALS

INTRODUCTION

Historical Review

Nearly two hundred years ago, in 1773, Peyrilhe performed perhaps the first experiment on the transplantation of tumors, "To form such inquiries on the causes of cancerous virus, as may lead us to ascertain its nature and effects, and the best methods of obviating it" (Preface, p. III). He injected material from the cancerous lesion of a human breast by syringe under the skin of a dog. Five days after the injection, "The whole skin, from the head to the tail, was completely emphysematous:—A little ichorous, blackish matter flowed from the wound.—The eyes of the animal were vivid, and he seemed to have great thirst: in this state the poor creature was perpetually howling . . . At length my maid, disgusted by the stench of the ulcer, and softened by the cries of the animal, put an end to his life, and thus prevented my observing the ultimate effects of this disease" (p. 46).

Many other attempts were made to transplant tumors from man to animals, but they consistently failed. Leidy, in 1851, transplanted human tumors under the skin of frogs. The grafts became vascularized and in one case tumor cells persisted at the site of inoculation, but no progressively growing tumors developed. A long period of time was to elapse between these early experiments on heterologous transplantation of human cancer and those of more recent date, in which human cancer has been successfully transplanted into laboratory animals. Peyrilhe would have been less discouraged over the outcome of his abortive attempt to transplant human cancer could he have foreseen the difficult problems his successors had to overcome in searching for our present knowledge of the transplantability of tumors.

The acquisition of knowledge of the histologic appearance of various malignant tumors in man, begun by Schwann and by Müller in the eighteen thirties and greatly advanced by the application of Virchow's principles of cellular pathology, gradually replaced the theory of humoral pathology that had dominated medicine for many centuries. Upon this new foundation was built the concept that small emboli are carried in lymph and blood vessels from the site of the primary cancer to distant organs, where they lodge and form metastatic tumors having the same histologic pattern as the primary tumor. The fact that cancer cells can be transferred from one site to another and then proliferate to form a tumor at the new site was made clearer by Cornil, who in 1891 described the successful experimental autotransplantation of human cancer from one breast to the other.

Advance beyond this point depended upon the recognition, of fundamental importance to the experimental study of cancer, that tumors occur in animals. Until 1858, when Leblanc called attention to the frequent occurrence of tumors in domestic animals, it was widely believed that cancer is a disease peculiar to man. Leblanc reviewed and appraised case reports of tumors in the horse, mule, donkey, cow, dog, cat, and pig and emphasized the importance of histologic examination in the diagnosis of tumors. McFadyean, in 1890, and Sticker, in 1902, published extensive data on tumors in animals. About this time the British Imperial Cancer Research Fund began an investigation of the distribution of tumors in the animal kingdom, and by 1904 Bashford and Murray could state definitely that cancer occurs in wild as well as in domestic animals. They believed that the disease in animals was essentially the same as it is in man and would probably be found to occur throughout the vertebrate phyla.

Attempts at transplantation concerned first spontaneous and then deliberately induced tumors of animals. Nowinsky, in 1876, described the transplantation of the venereal tumor of the dog. In nature, this tumor is passed from animal to animal during copulation, apparently by the implantation of tumor cells. It is the only instance outside the laboratory in which tumor cells are known to be passed from one animal to another.

In 1889 Hanau transplanted a squamous cell carcinoma from the region of the vulva of an old rat into other old rats of the same stock and carried the tumor for 2 transplant generations. He expressed the opinion that he had transplanted proliferating cells and not an infectious lesion. Von Eiselsberg, in 1890, and Firket, in 1892, were able to transplant sarcomas of the rat. In 1894 Morau carried a spontaneous mammary tumor of a mouse through several transplant generations, and in some cases he used hosts obtained by brother to sister matings. These transplantations received little notice. A review by Sailer, in 1900, lists some successes and many failures in the transplantation of tumors within animal species. Microscopic confirmation of the diagnosis of tumor was lacking in many cases, and uncertainty persisted as to whether cells or an infectious agent, variously suspected of being an animal parasite, a bacterium, or a mold, was transferred and gave rise to the new tumor. However, during the years 1901 to 1907, Loeb, Jensen, Borrel, Bashford and Murray, and Flexner and Jobling, made histologic studies and supplied convincing evidence that the transplantation of a tumor in experimental animals is dependent upon the continued multiplication of neoplastic cells from the original tumor. They demonstrated that tumor cells may reproduce indefinitely when transplanted serially under favorable conditions. The tumor cells of the Jensen Rat Sarcoma, growing today in host rats in many laboratories of the world, are the direct descendants of the cells of the original tumor which Jensen transplanted in 1908.

In the early experiments on transplantation it was customary to inoculate a spontaneous tumor into a large number of animals. The hosts as a rule were not related to the original animal that bore the tumor, and so the subsequent growth of the transplant was unpredictable. The tumor tissue might grow in none of the hosts or in only a few. Rarely did it grow in all the hosts into which it was transplanted. A large number of the tumors regressed spontaneously. As a result of the systematic charting of tumor growth, it was recognized about 1904 that mice in which transplanted tumors had regressed were immune to subsequent transplants of the same line of tumor and often to other tumors as well. According to Clowes, this discovery was principally responsible for the expansion of cancer research throughout the world.

Although Nowinsky, Hanau, and Morau had a vague notion that transplanted tumors grew better in kindred animals, it remained for later investigators to prove the importance of a close blood relationship between donor and host animals. In 1903 Jensen transplanted a cancer that originated in a white mouse to other white mice and to gray mice. The tumor grew in 40 to 50 percent of the white hosts for 19 generations. However, it grew in fewer gray mice, and the rate of growth was slower. In 1904 Loeb transplanted a submaxillary gland tumor of a Japanese waltzing mouse to other Japanese waltzing mice for 2 generations, but he terminated the experiment because it was difficult to procure a sufficient number of the Japanese mice. Loeb drew attention to the variations existing among individuals or families of the same species that may influence the growth of tumors.

Between 1909 and 1916 Tyzzer and Little attempted to identify factors responsible for the differences in susceptibility and resistance to transplantable tumors with respect to the genetic constitution of inbred, hybrid, and backcross mice. The development of inbred strains began with Little's brother to sister mating of dilute brown mice and the inbreeding of other races of mice by Bagg and by Strong. This early work has been reviewed by Strong and also by Heston. Nearly 100 inbred strains of mice are now in existence. Inbreeding reduces to a minimum the variations that are present among individuals of a heterogeneous population and thereby facilitates the transplantation of tumors. In 1924 Little and Strong formulated a genetic theory of transplantation. Briefly, the outcome of transplantation depends upon the degree of genetic similarity between the grafted tissue and the host. A cancer originating in one member of an inbred strain usually grows readily when transplanted to other members of the strain or to first generation hybrids. Tumors originating in these hybrids usually cannot be grown in the parent strain, since neither contains all the genetic factors present in the hybrids. The rate of growth of a transplanted tumor, its spread, its physiologic effects on the host, and its killing time generally show little fluctuation in inbred animals. Moreover, the pattern of spontaneous cancer in each inbred strain of animal is in general specific for age and sex as well as for site and histologic type.

Some transplantable tumors will grow in heterozygous animals or in unrelated inbred strains of the same species, despite genetic differences between donor and host. All the well known transplantable tumor lines developed during the first two decades of this century are, in fact, lines capable of transplantation in heterozygous hosts. The Brown-Pearce Tumor of the rabbit, for example, has been transplanted in strains of rabbits in which brother to sister mating has not been practiced. Under these conditions the success of transplantation is often variable and regressions may be frequent. Sarcoma 37, which arose in a non-inbred mouse, was for years transplanted to market and stock mice. However, this tumor may grow better in mice of some inbred strains than of others. With time, Sarcoma 37 may become so well adapted to a given inbred strain that the percentage of successful transplants is much higher than in strains into which the tumor is transplanted for the first time.

In general, transplantation is successful only within the species in which the tumor originated (homotransplantation), although certain measures have been devised whereby a tumor can be made to grow in alien species (heterotransplantation). In 1912 Murphy found that the cells of certain tumors of the mouse and rat would grow when explanted to the chorioallantoic membrane of the chick embryo, and that these tumor cells could be maintained by continuous passage from egg to egg. Since that time, tumors from chickens, ducks, mice, rats, rabbits, guinea pigs, and man have been explanted to the fertile egg by the use of the chorioallantoic membrane or the yolk sac, or by the injection of tumor cells into the blood stream or into the substance of the embryo. The developing chick embryo shows little or no resistance to the growth of heterologous tumor tissue and will supply blood vessels and connective tissue for its support. Since some tumors can be carried by continuous passage in the chick embryo, it is possible after a few egg passages to obtain a pure strain of tumor cells devoid of stroma from the original tumor. This procedure has been found useful for immunological studies. Tumors of the mouse, rat, chicken, and man growing in the egg are being used to assay the tumor-inhibiting activity of various agents.

Embryonic, immature, and newborn animals in which immunity has not reached its fullest development have been used to accomplish heterologous transplantation. Bullock, in 1915, described the successful transfer of one sarcoma and two carcinomas of mice to newborn rats. Putnoky (1938) and DeBalogh (1940) propagated virulent strains of the Ehrlich Carcinoma of the mouse in young rats and found that the tumor killed approximately 60 percent of the animals within two weeks. In 1950 Patti and Moore grew Sarcoma 180 of the mouse by transplanting it intraperitoneally to newborn rats. The rats died in about a week with widespread intra-abdominal tumor and pulmonary metastases.

Partial abrogation of resistance to heterologous tumor transplants has been achieved by producing acquired tolerance following the methods of

Billingham and associates. The inoculation of whole blood and leukocyte fractions from donor mice of different strains into embryo mice altered the response of the injected mice so that the survival of skin grafts from the donor mice was greatly prolonged. Application of the principle of acquired tolerance has led to the adaptation of strain or species specific tumors to grow in hitherto insusceptible hosts (Koprowski et al.). For example, Swiss mice were made tolerant to the strain-specific 6C3HED Ascites Tumor by prenatal inoculation of blood from C3H mice or of cells of the 6C3HED tumor itself. In the latter instance, the tumor grew in the fetus and continued to grow in the Swiss mouse after birth; subsequently this tumor was readily transplantable in adult untreated Swiss mice. A similar technic was applied to the DBA Mouse Lymphoma and the AH130 Rat Hepatoma. The tumors that grew in the baby Swiss mice after inoculation of tumor cells in utero were not readily transplantable to Swiss mice in the ascites form, but after several intracerebral passages in baby Swiss mice, they grew progressively as ascites tumors in untreated adult mice of this strain. In this way, several lines of the 6C3HED DBA and AH130 tumors were adapted to grow in Swiss mice. The adapted lines resembled the original tumor in immunological properties, but the cell volume, the DNA and RNA content, and the chromosome modality were increased. The original tumors were insusceptible to the action of several oncolytic viruses, while the adapted lines were readily destroyed by infection with these agents.

The resistance of adult animals to transplantable tumors may be diminished by blockage of the reticuloendothelial system with particulate matter, by exposure to roentgen rays, and by treatment with cortisone. Toolan, in 1951, reported that she had been able to grow human tumors and some normal tissues in previously irradiated rats. The transplants regressed, however, after the rats recovered from the effect of irradiation. In 1952 Green and Whiteley succeeded in transplanting a pulmonary carcinoma from man into the subcutaneous tissue of five cortisone-treated mice. That same year Sommers and associates published a series of papers in which they described the successful transplantation of human cancer in the subcutaneous tissues of the irradiated rat, in the cheek pouch of the untreated hamster, and on the chorioallantoic membrane of the chick embryo. In 1953 Toolan propagated human tumors in the subcutaneous tissue of cortisone-treated rats and in the cheek pouch of cortisone-treated hamsters. Some tumors were thus maintained in serial transplantation for more than two years. The cheek pouch was first utilized as a site for tumor transplantation by Lutz and associates.

The brain and the eye are not much affected by the immune response of the host, and hence heterologous transplant tumors will often grow in these sites in untreated adult animals. In 1938 Greene reported progressive growth for more than 80 days of human mammary carcinoma transplanted into the anterior chamber of the eye of the rabbit, and he later showed that heterolo-