Comparison of Concomitant and Sinecomitant Tumor Immunity[1] (34989)

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Demonstration of tumor specific immunity has, for the most part, been carried out experimentally by evaluating resistance to a tumor challenge after prior exposure of animals to the same tumor (1–4). Progressive growth of such immunizing tumors has been prevented (for the most part) by their ligation or excision. Immunity in animals with growing tumors, which was designated by Bashford *et al.* in 1908 as "concomitant immunity (5)," has received less attention. Evidence in support of its existence is meager (6–8), and since early studies of this phenomenon were carried out in nonisogenic systems, their significance is dubious (9, 10).

Because of the paucity of information relative to the presence of concomitant immunity in isologous tumor–host systems, it was considered worthwhile to determine its presence and to compare the degree of such immunity, if it existed, with that which occurred after removal of an immunizing tumor. To differentiate between the two, the term "sinecomitant" immunity is used to designate that tumor immunity which exists in a host without a growing tumor in contrast to "concomitant" immunity which denotes immunity in the presence of a growing tumor.

Materials and Methods. Tumor plugs measuring 1 × 0.5 mm were implanted subcutaneously in the abdomen (rat) or left hind leg at the ankle (mouse). Resulting tumors were allowed to grow undisturbed for the demonstration of concomitant immunity. Sinecomitant immunity was produced by complete removal of the transplant after 14 days of growth by excision in the rat and by amputation of the tumor-bearing leg below the knee in the mouse. The presence or absence of immunity was determined by the growth of a suspension of cells prepared from the same immunizing tumor inoculated subcutaneously in the right hind leg. The cell suspension was prepared by passing finely minced tumor tissue through a cytosieve into Medium 199. Viability of cells was tested by trypan blue dye exclusion. Four experiments were carried out in the rat, employing challenge doses of 1×10^6, 5×10^5, 2.5×10^5, and 2.0×10^5 tumor cells. Two experiments were performed in mice using 1.0×10^4 and 1.5×10^4 cells as the challenge. All animals were examined daily for appearance and subsequent growth of tumor. Experiments were terminated 35 days after challenge.

Studies with MC tumor in the rat. Young adult inbred (Lewis) females averaging 200 grams in weight, maintained in individual cages and fed standard laboratory chow and tap water were employed. The tumor used was produced in a female Lewis rat by the subcutaneous injection of methylcholanthrene (MC) in sesame oil. It was maintained by serial passage and was in the 7th to 10th generation when used.

Animals were randomized into four groups. Those in Group I were not immunized with tumor prior to challenge and served as controls. Rats in Group II were challenged 14 days after complete removal of the tumor which had been present for 14 days (sinecomitant immunity). Groups III and IV, which were composed of rats with a 14-day-old tumor at time of challenge, were randomized 7 days after challenge. In Group III, the immunizing tumor was permitted to grow without interference (concomitant immunity). Interrupted concomitant immunity was produced in Group IV by complete removal of

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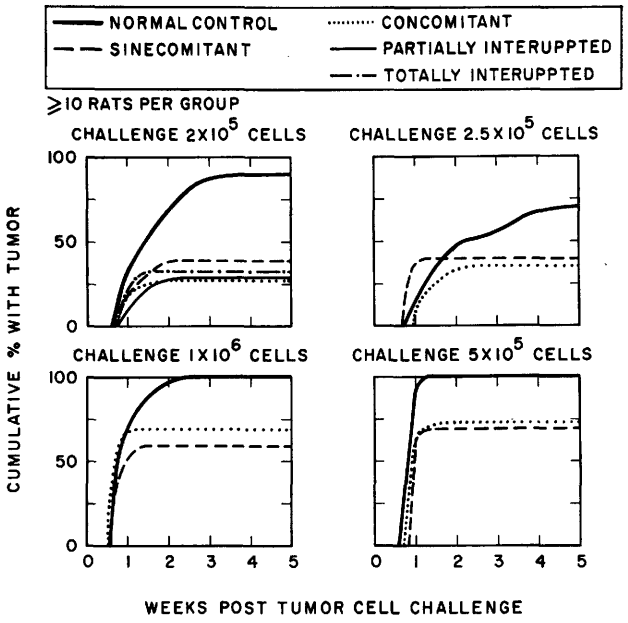

FIG. 1. Comparison of concomitant and sinecomitant immunity in rats to MC tumor.

the tumor at 1 week or partial removal at 1 and 3 weeks after challenge. At the time of excision, the greatest tumor diameter averaged 4.5 cm. A portion of tumor approximately 1 cm in diameter remained after partial excision.

Studies with spontaneous tumor in mice. Eight-week-old inbred (C3HeB/FeJ) mice were employed. They were housed and fed as were the rats. A spontaneous C3H mammary carcinoma was used prior to its 10th transfer generation.

Animals were randomized into four groups. Those in Group I, having no exposure to tumor prior to challenge, served as normal controls. Their left hind legs were amputated 3 weeks before challenge. Mice in Group II had tumors growing for 14 days, and which were amputated 3 weeks before challenge (sinecomitant immunity). Mice in Groups III and IV, bore 14- and 3-day-old tumors at time of challenge, and such tumors were permitted to grow undisturbed after challenge (concomitant immunity).

Results. Comparison of concomitant and sinecomitant immunity in rats (Fig. 1). Immunity to a transplanted MC tumor was demonstrated by each of the four different challenge doses employed. This was most apparent when a smaller tumor cell inoculum was used (2×10^5 cells). Sinecomitant, concomitant, and interrupted (either partial or complete) concomitant immunity were essentially of the same magnitude. At 35 days, for example, whereas 9 of 10 normal rats (90%) injected with 2×10^5 cells had tumors growing, only 4 of 10 (40%) which had a tumor removed 2 weeks prior to challenge demonstrated tumor. When tumors were present at challenge, and were subsequently undisturbed, 3 of 11, or 27%, had growth of the tumor challenge. When tumors were reduced in size 7 and 14 days after the challenge, growth was similar to that in animals with undisturbed primary tumors, being 30% (3 of 10) for each group. Tumors growing in immune animals failed to differ from those in controls relative to time of appearance and subsequent growth rate.

Comparison of concomitant and sinecomitant immunity in mice (Fig. 2). Growth of a tumor cell inoculum was similar, whether the tumor was present during and after challenge, or it had been removed 2 weeks previously. In both circumstances, employing two different challenge doses the incidence of tu-

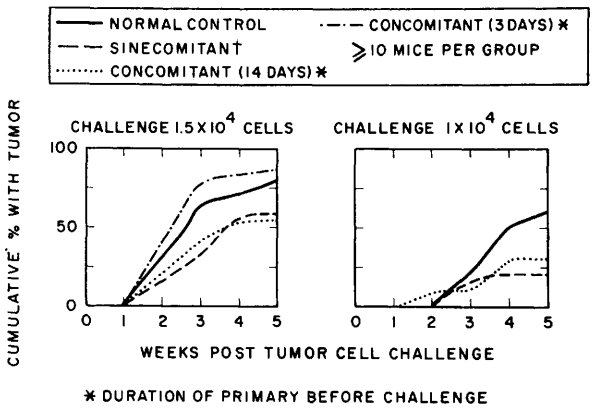

FIG. 2. Comparison of concomitant and sinecomitant immunity in C3H mice.

mors occurring was less than in animals having no prior exposure to tumor. When, for example, 1×10^4 cells were used as a challenge, 6 of 10 (60%) normal mice grew tumor. Only 3 of 12 (25%) whose immunizing tumors had been removed, and 3 of 10 (30%) with growing tumors developed them. The presence of a tumor for only 3 days prior to challenge failed to evoke immunity which inhibited growth of the challenge.

Discussion. While tumor-specific immunity in animals exposed to but no longer harboring a tumor has been firmly established, such immunity in those with progressively growing tumors has been less convincingly demonstrated. Riggins and Pilch (11) observed immunity in C3H/HEN mice to a spontaneous adenocarcinoma as well as to an MC-induced fibrosarcoma when tumors were allowed to grow temporarily in a hind limb, but were unable to demonstrate any resistance to tumor challenge in animals from which the immunizing tumor was not removed. Such findings were in keeping with those of Foley (1) who had previously noted in C3H/HEN mice, that MC-induced sarcomas implanted prior to ligation of the immunizing tumors grew, but that growth did not occur if ligation had been carried out prior to challenge. Mikulski *et al.* (12), tested the ability of cells isolated from benzopyrene-induced sarcomas in rats to grow when injected back into autochthonous hosts. Such autografts did not take if the original tumor had been removed, but grew in animals in which the major part of the tumor remained. The same authors observed (13) that the growth rate of chemically induced tumors in syngeneic hosts was retarded if tumor cells had been mixed with spleen cells from a syngeneic donor which had been immunized against the tumor by injections of lethally irradiated tumor material. A protective effect was not, however, achieved if spleen cells were obtained from animals bearing large tumors. Gershon and Carter, on the other hand, observed (14) that spleen cells and peritoneal exudate cells from hamsters with large tumors were effective in suppressing the growth of homologous tumor cells in syngeneic recipients.

Old and Boyce, employing transplanted chemically induced tumors in isogenic hosts reported that they observed such immunity (6). No data were presented. Recently Gershon and associates (7) noted that hamsters grafted with an allotransplantable lymphoma were refractory to reinoculation with cells of the same tumor although the initial graft grew progressively. Moreover, removal of the tumor prior to challenge led to a rapid decrease in immunity (15). Bard *et al.* (8), have observed that when C3H mice with growing transplanted MC-induced tumors were challenged with the same tumor, animals manifested resistance to growth of the challenge.

This study demonstrated the existence of concomitant immunity in both rats harboring transplanted MC-induced tumors and in

mice with transplanted mammary tumors. The latter is, to our knowledge, the first demonstration of such immunity to a non-chemically induced syngeneic tumor in isologous animals. Moreover, such immunity was equivalent to that found in animals from which prior growing tumors had been removed. It was also of interest that either partially or totally removing the immunizing tumor 7 days after exposure of the host to a challenge failed to either enhance or depress the immune response.

The findings that, despite the inhibition of secondary tumor growth, primary tumor continued to grow, indicates that mechanisms other than enhancement, inadequate antigenic stimulation and impaired immune response are necessary to explain the progressive growth of the latter. Perhaps as Gorer *et al.* have suggested (16), the tumor is largely inaccessible to cell-bound antibodies (*i.e.*, lymphocytes) or the magnitude of the immune response evoked is inadequate to inactivate the vast number of cells present in the primary tumor.

Demonstration of equivalence of sinecomitant and concomitant immunity is of importance to those engaged in such studies in the laboratory. For, aside from shortening the time and simplifying the methodology for production of immune animals, a model utilizing concomitant immunity more closely simulates the human situation.

Summary. Concomitant immunity has been observed in syngeneic rats (Lewis) harboring a progressively growing methylcholanthrene-induced tumor, and in mice (C3H) with spontaneous mammary tumors. Such immunity, as determined by the growth of a challenge of tumor cells from the immunizing tumor, was equivalent to that found in animals whose primary tumors had been removed prior to challenge (sinecomitant immunity). Partial or complete removal of the primary tumor subsequent to challenge failed to alter immunity.

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