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Immunologic and Chemotherapeutic Effects on Human Melanoma Heterotransplants.*† (31591)

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Lymphocytes have been known to inhibit or restrain tumor and other tissue transplants since the experiments of Murphy in 1926(1). The sequential transplantation of human cancers in hamster cheek pouches usually requires preconditioning by cortisone or irradiation, which are thought to repress lymphocyte-borne immune responses. In the present experiments human melanoma (ME-1) transplants grew larger in cortisone preconditioned hamsters that were treated with antilymphocyte serum. Conversely, immunologically enhanced rejection of tumors was achieved by pretreating hamsters with footpad injections of frozen tumor and Freund's adjuvant.

Methotrexate treatment significantly reduces the growth of the ME-1 tumor(2). The therapeutic effect persisted when treated tumors were subsequently transplanted into untreated hamsters.

Materials and methods. A human malignant melanoma (ME-1) previously studied in other investigations was transplanted, utilizing the same technique, size of male hamsters, diet and amount of cortisone as already reported(2,3).

To prepare antilymphocyte sera, the axillary, cervical and mesenteric lymph nodes of normal hamsters were mechanically minced, suspended in physiologic saline solution, and the mixture filtered through wire mesh. The washed lymphocytes were mixed with equal volumes of incomplete Freund's adjuvant, without a bacterial component. From 20,000 to 100,000 lymphocytes were present per ml of saline. The antigen was administered to rabbits, 1.5 ml subcutaneously, once a week for 4 injections. The animals were bled for antiserum 7 to 10 days after the last injection.

Antilymphocyte activity of the rabbit antisera was tested by injecting 1 ml intraperitoneally into normal hamsters. Single injections reduced the blood lymphocytes and monocytes from an average of 65% to an average of 33% for a period of 6 days thereafter. Attempts to quantitate the changes further by lymphocyte counts were unsuccessful, due to large variations in numbers of blood lymphocytes in both control and antiserum-injected hamsters. Tests of the antisera by precipitin and complement-fixation methods, using lysed lymphocytes as antigen, showed no reactions.

One intraperitoneal injection of 1 ml rabbit antiserum was given to 25 tumor-bearing hamsters on the fourth day after tumor implantation, in order to produce a maximal effect at the beginning of the logarithmic (log) phase of tumor growth(2).

Methotrexate was administered as previously reported, 10 mg intraperitoneally, to transplant donors, from which tumors were removed after 21 days of growth. These harvested tumors were implanted into 13 hamsters preconditioned with cortisone as usual, but not otherwise treated.

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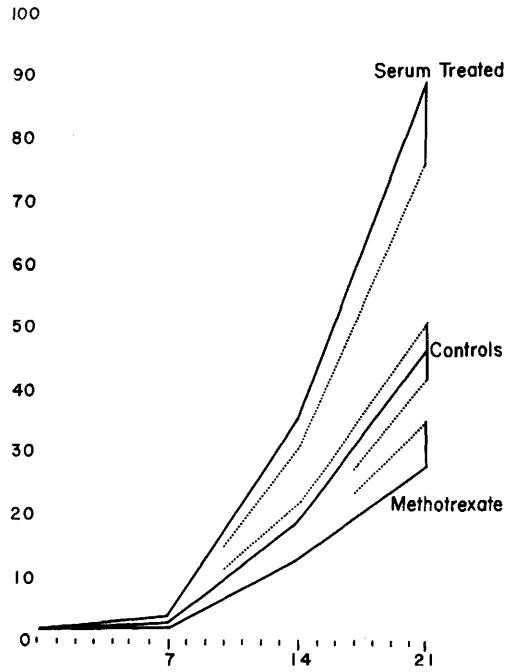

FIG. 1. Relative sizes of tumor growths in mm^3 of human melanoma are shown in animals treated with antilymphocyte serum, in cortisone conditioned controls and in transplants of tumors pretreated with Methotrexate. Abscissa represents days after tumor transplantation.

Footpad injections were made of ME-1 tumor and adjuvant. Excised tumor was minced, mashed through wire screen, diluted with physiologic saline, mixed with equal volumes of incomplete Freund's adjuvant and kept frozen until use. Depending on the hamster's size, either 0.05 or 0.1 ml of the mixture was injected twice weekly, each time into a different footpad, for a total of 6 injections. On the 20th day after the first injection, ME-1 tumor was implanted in 43 experimental and 39 control hamsters, to which treatment was given as usual thereafter. Control animals were injected in the same way with mixed normal saline and incomplete Freund's adjuvant, and subsequently transplanted with tumor, using the same techniques.

The means and standard deviations of measured tumor volumes were recorded graphically. Differences between groups were evaluated statistically by determination of coefficients of regression, and probability (p) values less than 0.05 were considered significant. Chi-square tests were used to compare the groups with tumor growth and tumor rejection after one, two and three weeks following transplantation.

Results. On antilymphocyte-serum treated hamsters ME-1 tumors grew considerably larger than in controls (Fig. 1 and Table I), notably between the 7th and 21st days after transplantation, during the logarithmic (log) phase of tumor growth. The relative increase was significant both at 14 days and 21 days after transplantation ($p<0.005$). No histologic differences were found between the groups.

Methotrexate treated donor tumors grew more slowly in untreated hosts than in controls (Fig. 1 & Table I). In the third week after transplantation the differences were statistically significant from the 18th to the 21st days ($p<0.05$). The treated and control groups showed no histologic differences.

After footpad injections of the tumor and Freund's adjuvant there were fewer successful tumor transplantations. Differences were not significant in the first week, since 42 of 43 pretreated hamsters and 38 of 39 controls had successful transplants. In the second week after tumor implantation, only 19 of 39 pretreated animals had tumors present, compared to 33 of 38 controls ($p<0.005$). In the third week, 16 of 38 pretreated hamsters and 25 of 33 controls had tumors ($p<0.01$).

Transplanted ME-1 tumors in pretreated hamsters were slightly smaller than in controls during the first week. Later the pretreated animals with successful transplants had tumors of too wide a range of size for statistical analysis.

Discussion. Successful prevention or conversely enhancement of tumor transplant growth has been previously achieved experi-

TABLE I. Transplanted Tumor Volumes in mm^3 in Hamsters Treated with Antilymphocyte Serum, Donors Treated with Methotrexate and Controls.

Days after transplant	Serum (24 animals)	Methotrexate (13 animals)	Controls (29 animals)
7	4.9 ± 2.52	1.91± .84	3.35± 2.16
14	35.2 ± 4.22	13.1 ± 2.80	19.1 ± 2.90
21	89.6 ±12.9	28.1 ± 7.05	46.5 ± 5.62

mentally by immunologic or chemical means (1,2,4). Usually homologous rather than human neoplasms have been employed. Lymphocyte-borne immunity probably represents the first recognized host reaction induced by foreign tissues, including neoplasms(1).

Cortisone preconditioning and irradiation have made heterologous tumor transplantation into hamsters successful allegedly partly because they have inhibitory effects upon host lymphocytes. But host resistance appears incompletely inhibited by cortisone, since the growth of transplanted human melanoma was enhanced after antilymphocyte sera. The injection of antiserum was given at the critical time when tumor was beginning the logarithmic growth phase. Lymphocytes were decreased for a period of only 6 days, but this was sufficient for increased tumor growth.

Tumor transplant growth was often prevented by prior footpad injections of frozen melanoma and adjuvant. Transplant rejection occurred more frequently than restricted tumor growth. Some successful transplants under these conditions suggested that the enhanced host resistance might later be overcome by cortisone conditioning.

Independent of immune reactions, melanoma growth could be intrinsically altered by Methotrexate in subsequent untreated transplantations. Chemotherapy clearly affected the melanoma cells rather than the hamster lymphocytes. The alteration was functionally but not morphologically demonstrable. These experiments suggest that a more effective control of some neoplasms might be achieved by therapy directed simultaneously at enhancing host resistance and reducing tumor growth activity.

Summary. Human malignant melanoma grew significantly larger than usual in hamsters treated with antilymphocyte sera. Footpad injections of frozen tumor with adjuvant resulted in increased melanoma transplant rejections. Methotrexate chemotherapy of donor melanoma reduced the subsequent growth in untreated hosts.

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Distribution of Virus-Like Particles in the Lymphatic Tissues of "Nonleukemic" CFW$_W$ Conventional Mice.* (31592)

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Virus-like particles have been described not only in leukemic mice(1-4) but also in "nonleukemic" conventional and germfree mice(5-7). Most of the particles indicated in these reports were located in the thymus. The purpose of this study is to compare the particle distribution in the thymus with the spleen and lymph nodes of "nonleukemic" conventional CFW$_W$ mice.

Materials and methods. The mice used in this study were randomly inbred CFW mice raised locally for 8 years and designated as CFW$_W$ mice. An examination was made of 18 uninoculated conventional CFW$_W$ mice ranging in age from 1 to 6 months. In our laboratory these conventional mice show a 19% spontaneous incidence of lymphocytic leukemia by 12 months and 29% by 24 months of age. All the uninoculated animals

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