# Immunity of C3H Mice to Lymphosarcoma 6-C3H-Ed Following Regression of the Implanted Tumor. (19728)

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Immunity in mice and rats against tumors which arose in and which grow progressively in certain pure bred lines of these animals, following atrophy of the tumor, has been observed by a number of workers. MacDowell (1) by repeated injections of small numbers of leukemia cells (which failed to induce leukemia) was able to immunize C58 mice against a transplantable leukemia specific for this strain. Gross(2,3) implanted C3H mice intradermally with small quantities of a methylcholanthrene induced sarcoma which arose in this strain, many of the tumors regressed, and mice which had borne the regressive tumors were immune to subsequent subcutaneous implantations of the same tumor. Goldfedder (4) described immunity of inbred Bagg rats to a transplantable lymphosarcoma which arose spontaneously in this strain, which was induced following implantation of fragments of the tumor which had been attenuated by x-rays. More recent mention has been made (5) of similar experiments carried out with a spontaneous mammary tumor which arose in Bittner's high cancer line of mice, in which implantation of x-rayed tumor tissue induced immunity in autogenous hosts. Stoerk and Emerson(6) induced severe riboflavin deficiency in C3H mice bearing lymphosarcoma 6-C3H-Ed, the tumors regressed and those mice which survived the vitamin deficiency were found to be immune to subsequent implantation of the tumor. Lewis *et al.*(7) by use of a variety of technics were successful in impairing the blood supply of transplantable sarcoma which arose, and were progressive upon transplantation, in pure bred rats of the Lewis and King A strains. The treatments caused atrophy of the tumors which resulted in immunity of the rats to subsequent implantation of the same tumors. They further demonstrated(8) that sarcoma tissue undergoing autolysis as a result of being sewn into a skin pocket, when transplanted into other rats of the same strain induced immunity against implantation of the tumor. We have observed that a number of Jax-C3H-(He) mice in which regression of lymphosarcoma 6-C3H-Ed was induced by intensive treatment with A-methopterin were immune(9). The present paper describes an extension of these experiments and others in which Jax-C3H mice of the Heston subline were immunized by means which prevented or interrupted the progressive growth of the tumor in these mice.

*Materials and methods.* Young adult (18-22 g) C3H (Heston subline) mice of both sexes and the Gardner lymphosarcoma 6-C3H-Ed, obtained from the Jackson Memorial Laboratory were used in all experiments. The tumor was carried for over 70 passages in these mice in our laboratory within the last 2 years, and in the passages and in numerous experiments in which these mice were implanted with it for control purposes, it has not failed to grow progressively and kill the recipients. The tumor was implanted by subcutaneous injection of tumor cell suspensions made by mincing 10-12-day-old tumors with scissors in saline. The suspensions were of such density that passage through a No. 22 gauge needle was accomplished. The mice were observed almost daily and were kept under the usual laboratory conditions, having a free access to food and water. A-methopterin was injected subcutaneously in 2 mg/kg/day doses of aqueous solution. Other methods are described in connection with the various experiments.

*Experimental.* In 6 experiments a total of 62 Jax-C3H (He) mice were implanted on the right flank with tumor cell suspensions. The dose implanted ranged in the various experiments from approximately 250,000 to 5,000,000 cells in 0.1 ml of saline. Beginning on the first to the fourth day following implantation, 2 mg/kg/day doses of A-methopterin were given. Dosage was continued for 7 successive days in recent experiments, and for 11

days in others(9). Two mice died during treatment, and of the 60 mice which survived, the tumor regressed in 21, usually between the 20th and the 35th day following implantation. Thirty-six untreated mice similarly implanted as controls died of progressive tumors between the 18th and 30th days depending upon the number of cells implanted. The 21 mice in which progressive tumor growth failed to occur were reimplanted with approximately 5 million tumor cells 6 or 7 weeks after the original implantation. Eleven of these mice which were not immune had never developed palpable tumor following the first implantation, whereas all of the 10 which were immune were mice in which regression of palpable tumors had taken place under the influence of A-methopterin. It is inferred from these observations that the tumor mass acted as the antigenic stimulus in inducing immunity.

Five Jax-C3H (He) mice were slowly injected into the tail with approximately 2.5 million tumor cells in 0.05 ml of saline suspension, care being taken to miss the tail veins. The injection was made equidistant between the base and the tip of the tail. All 5 of the mice failed to develop visible tumors. Four weeks after the implantation, the mice were challenged for immunity by subcutaneous injection of approximately 5 million tumor cells. Four of the mice failed to develop tumors, the fifth developed a pea size tumor which regressed. Six control mice injected with the same tumor cell suspension died of progressive tumors. The 5 immune mice were rechallenged after 2 weeks and again tumors failed to grow. The experiment was repeated using larger numbers of cells for implantation.

Twenty-five Jax-C3H (He) mice were injected into the tail with 5 million tumor cells in 0.1 ml of saline suspension. Eleven of these developed tumors in the tail which spread to the rump, killing the mice on or before the 42nd day after injection. Fourteen mice failed to develop tumors and were challenged on the 42nd day by subcutaneous injection of 5 million tumor cells into the flank. These 14 mice failed to develop tumors while each of 15 controls died of progressive tumors within 3 weeks after injection.

Eight Jax-C3H (He) mice were implanted in the same location in the tail as those described above. Approximately 10 million tumor cells were injected in 0.2 ml volumes of saline. Considerable force was used in injecting the dose and some oozing of fluid through the skin of the tail was observed. After 12 days, all of the mice had developed tumors which extended 0.25 inch in either direction from the point of injection. At 5 P.M. the blood supply of the tails was occluded by winding short lengths of No. 14 A.W.G. soft drawn copper wire around the tail about 0.5 cm from its base. The wire was made tight and secure by twisting the ends together with hemostats. Almost immediately the tail became engorged and the tumors discolored to a deep purple. The wires were left in place overnight. The next morning the tails were dry and blackened and the tumor severely damaged, being swollen, black and exuding thin cherry red liquid through fissures which had developed during the night. The tails and tumors retained this appearance even after the wires were removed at 9 A.M. The process was repeated at 5 P.M. on the same day with removal of the wires the following morning. At this time the tails and tumors were distinctly dry, and later in the day the lower portion of the tails were observed to have fallen off, breaking in the region where the tumors had been. No further treatment was given and the mice were observed for 10 days during which period one died. The others remained well although the tails became shorter, finally breaking at the point where the wires had been applied. Six weeks after the original implantation, these mice and 6 controls were injected subcutaneously in the flank with approximately 5 million tumor cells. Within 5 days pea size tumors developed in all of the mice. The tumors in the control mice grew progressively, causing death, while those in the test mice after remaining pea size for 4 days began to regress and all had disappeared by the 15th day. A second challenge implantation of tumor cells was given one month after the first; the tailless mice developed no tumors while each of 5 controls died with progressive tumors. The results of the experiments are combined and summarized in Table I.

TABLE I. Immunity to Lymphosarcoma 6-C3H-Ed Produced in C3H (He) Mice by Prior Implantation of the Tumor.

| Experimental procedure | No. mice | Progressive tumors | Palpable tumors regressed | No. tumor developed | Total negative to first tumor implantation | Result of reimplantation of tumor | |
|---|---|---|---|---|---|---|---|
| | | | | | | Not immune | Immune |
| A-methopterin, 2 mg/kg/day for 7 days | 62 (2)* | 39 | 11 | 10 | 21 | 11 | 10 |
| Tumor inj. in tail (no treatment) | 30 | 11 | | 19 | 19 | | 19 |
| Tumor inj. in tail (tails tied off) | 8 (1)† | | 7 | | 7 | | 7 |

* This tabulation includes mice in experiments previously described(9).
† Number in parenthesis died during treatment.

*Summary.* Experiments are described in which cells of lymphosarcoma 6-C3H-Ed implanted in Jax-C3H (He) mice, in doses which failed to induce tumors when implanted into the tail, in large doses which induced tumors that were destroyed by intermittent occlusion of the blood supply, and by regression of palpable tumors through intensive treatment with A-methopterin, led to development of immunity of the mice against subsequent progressive growth of the same tumor.

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## The Mast Cell. Cortisone Action on Connective Tissue.* (19729)

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During recent years the effect of hormones on connective tissue has been the object of intensive research, particularly after it became known in 1949 that the adrenal cortex influenced a number of connective tissue diseases.

It is known(10,11) that cortisone inhibits new formation of connective tissue and that fibroblasts in healing wounds of treated individuals are smaller and more pyknic than in wounds of untreated subjects.

The effect on the connective tissue ground substance has attracted particular attention, because the formation of ground substance appears to be the essential condition for new formation of connective tissue. Since various observations(1,2,5) indicate that hyaluronic acid, which is an important component of the connective tissue ground substance, is formed by the mast cells, interest has also centered on the effect of hormones on these cells.

The present author(3,13) found that in

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