Effect of Cortisone Acetate on Growth of Strain Specific Tumors in Alien Strains of Mice. (19726)

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In a previous publication(1) experiments were described in which CF_1 mice implanted with lymphosarcoma 6-C3H-Ed (originating in C3H mice) and treated with cortisone acetate died of progressive growth of the tumor. Similar observations were made by Howes(2) who described progressive growth of adenocarcinoma EO771 and 775 (originating in C57 mice) when transplantations were made to Rockland Swiss mice treated with cortisone acetate. The present report describes further studies on the effect of cortisone acetate in promoting the growth of lymphosarcoma 6-C3H-Ed in CF_1 mice and attempts to induce progressive growth of this and other strain specific tumors in other alien strains of mice treated with cortisone acetate.

Materials and methods. CF_1 and AKM mice were obtained from Carworth Farms and ZBC mice from the surplus stock of Dr. J. J. Bittner. C3H (He), DBA-2, C57 Black, A (Lilly), and A (He) mice and all of the tumors except the Patterson lymphosarcoma, which was supplied by Dr. C. C. Stock, were obtained from the Jackson Memorial Laboratory. Routine passage of the tumors was made in mice for which the tumor is specific, (lymphosarcoma 6-C3H-Ed and adenocarcinoma C3H-BA in C3H (He), lymphatic leukemia P-1534 in DBA-2, adenocarcinoma EO771 in C57 Black and the Patterson lymphosarcoma in AKM mice). Young adult mice of both sexes were used. In most experiments, cortisone was given subcutaneously as an aqueous suspension of the acetate in 0.25 ml volumes. The animals were palpated 3 times a week to determine growth behavior of the tumor. Additional details are given in connection with the experiments.

Experimental. CF_1 mice implanted with lymphosarcoma 6-C3H-Ed taken from C3H mice develop large tumors which reach their maximum size in about 2 weeks, after which rapid regression (usually complete in 2-4 days) takes place. Such mice are immune to reimplantation of the tumor. Similar immunity develops in CF_1 mice implanted with mixtures of spleen, lymph-node and thymus tissue from C3H mice. These observations suggested that lymphosarcoma 6-C3H-Ed grows progressively in cortisone treated CF_1 mice because such treatment interferes with the normal development of immune responses. The following experiment shows that the immunity which develops following implantation of lymphoid tissues from C3H into CF_1 mice is impaired by intensive treatment with cortisone acetate. Twenty CF_1 mice were implanted by trocar with fragments of spleen, lymph-node and thymus subcutaneously on the left flank. Beginning on the same day, 10 of the mice were begun on a course of treatment with cortisone acetate. Daily doses of 40 mg/kg were given for 7 successive days. Ten of the mice remained untreated. Ten days following implantation of the lymphoid tissue, all 20 mice and an additional 10 controls were implanted by trocar in the right flank with lymphosarcoma 6-C3H-Ed. The cortisone treated and the normal control mice developed large tumors, which in due course regressed; the mice implanted with lymphoid tissue and which received no cortisone failed to develop palpable tumors. These results are shown in Exp. 1 in Table I.

Experiments were made to determine whether the immunity developed by CF_1 mice in which lymphosarcoma 6-C3H-Ed had regressed could be destroyed by cortisone acetate treatment. Groups of such immune mice were treated according to different dosage schedules with cortisone acetate. In no instance was the immunity abolished, as is shown in Exp. 2 in Table I.

The finding that intensive treatment suppresses the development of the immunity of CF_1 mice induced by implantation of tissues from C3H mice (Exp. 1) suggested that serial passage of lymphosarcoma 6-C3H-Ed through groups of CF_1 mice treated with cortisone

TABLE I. Effect of Cortisone Acetate Treatment on Immunity of CF_1 Mice to Lymphosarcoma 6-C3H-ED.

Experimental procedure	Treatment	No. of mice	Immune to lymphosarcoma 6-C3H-ED
Exp. 1			
Implanted with a mixture of spleen, lymph-node and thymus tissue fragments from C3H mice	40 mg/kg/day for 7 days beginning on day of tissue implantation.	10	0*
	No treatment	10	10
Normal CF_1	" "	10	0
Exp. 2			
Mice immune as a result of regression of previously implanted lymphosarcoma 6-C3H-ED	40 mg/kg/day for 5 successive days. Tumor implanted 3 days after last dose was given.	8	8
	Treated for 7 days. Tumor implanted on day last dose was given.	8	8
	Treated for 3 days. Tumor implanted & treatment continued for 7 additional days.	13	13
	No treatment	8	8
Normal CF_1 mice		10	0

* Tumor implanted 10 days after C3H lymphoid tissue had been implanted.

acetate could be accomplished. To test this possibility, groups of 5 CF_1 mice were implanted subcutaneously by trocar with tumor removed from C3H mice. Cortisone injections (40 mg/kg/day) were begun within 4 hours after implantation and were continued for 7 successive days. Mice bearing large, obviously progressive tumors were killed between the 14th and 21st day and their tumors used for the next passage to new groups of CF_1 mice which were treated with cortisone acetate as were their predecessors. At each passage a control group of untreated CF_1 mice was implanted with the same tumor material in order to determine whether adaptation of the tumor to CF_1 mice had occurred. Two experiments were conducted. In the first, 4 serial passages were made before all mice in the treated group suddenly died; in the second, the tumor was passed in 9 successive groups of cortisone treated mice before the experiment was discontinued. In no instance did tumor taken from cortisone treated mice and transplanted into untreated CF_1 mice grow progressively. Apparently the species specificity of this C3H tumor was not altered by repeated passage in cortisone treated CF_1 mice.

The above results show that treatment with cortisone acetate impairs the development of the immunity of CF_1 mice induced by lymphoid tissue of C3H mice and it may be inferred that the progressive growth of lymphosarcoma 6-C3H-Ed in cortisone treated mice is a result of suppression of immune response to the C3H tumor tissue. Thus the "natural immunity" of CF_1 mice to lymphosarcoma 6-C3H-Ed which is manifest by regression may be related to (or identical with) specific foreign tissue immunity. In view of this, it was considered of importance to determine whether the resistance of other alien strain mice to progressive growth of this and other strain specific tumors could be abolished by intensive treatment with cortisone acetate. Table II shows the results of implantation of a variety of tumors into pure bred (DBA-2, C57, A and AKM) random bred, (CF_1) and backcross (ZBC) mice treated with cortisone acetate, together with data showing the effect of different dosage schedules on the progressive growth of lymphosarcoma 6-C3H-Ed in

TABLE II. Effect of Cortisone Acetate Treatment* on Progressive Growth of Various Tumors in Alien Strain Mice.

Implanted	Treatment with cortisone acetate	Recipient mouse	No. of mice	Growth of tumor: Progressive	Growth of tumor: No growth or regression
Lymphosarcoma 6-C3H-Ed	Normal untreated	CF_1	57	2	55†
	Mice pretreated for 3 days prior to tumor implantation	CF_1	10	0	10†
	Treated for 3 days beginning on the day of tumor implantation	CF_1	10	0	10†
	Treated for 10 days beginning on day of implantation	CF_1	30 (7)‡	17	6§
	Treated for 7 days beginning on day of tumor implantation	CF_1	62 (6)	37	19†
	"	DBA – 2	10	0	10
	"	C57 Black	10	0	10
	"	A (Lilly)	10 (1)	0	9
	"	AKM	10 (2)	0	8
Adenocarcinoma EO 771	"	ZBC	13	9	4
	"	CF_1	10	0	10
Leukemia P-1534	"	ZBC	10	0	10
	"	CF_1	10 (1)	0	9
Patterson lymphosarcoma	"	CF_1	10	0	10
	"	A (He)	10	0	10
Adenocarcinoma C3H-BA	"	CF_1	40 (5)	0	35

* Cortisone acetate aqueous suspension 40 mg/kg/day.
† Tumors grew to large size, then regressed completely.
‡ Number in parentheses indicates death during treatment.
§ Experiments previously described(1).

CF_1 mice.

Summary. Lymphosarcoma 6-C3H-Ed implanted in CF_1 mice intensively treated with cortisone acetate grows progressively, and adenocarcinoma EO-771 also grows progressively in ZBC mice under similar conditions. Neither of these tumors nor others tested grew progressively in other alien strains of mice treated with cortisone acetate. Progressive growth of tumors in alien strains of mice under the influence of cortisone acetate treatment is restricted to certain tumors in certain strains of mice, and is therefore not an expression of a general breakdown of natural barriers to tumor transplantation.

1. Foley, E. J., and Silverstein, R., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 713.
2. Howes, E. L., *Yale J. Biol. and Med.*, 1951, v23, 454.

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Resistance of C3H Mice to Lymphosarcoma 6-C3H-Ed Induced by Tissues from Mice of C3H Sublines. (19727)

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Immunity against tumors which grow progressively in strains of pure bred mice by prior injections of normal tissues from alien strains of mice has been described by MacDowell *et al.*(1), and by Rhodes and Miller(2), who studied leukemia, and by Barret *et al.*(3) using a fibrosarcoma. In experiments previously reported by us(4,5) it was shown that C3H mice,* obtained from the Jackson

* In the previous publications(4,5) these mice were referred to as Jax C3H mice. Specifically they are of the Heston subline maintained at the Jackson Memorial Laboratory and in the present paper are designated as Jax-C3H (He) mice.