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.

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

E. J. FOLEY. (Introduced by R. Tislow.)
From the Scientific Research Division, Schering Corporation, Bloomfield, N. J.

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.

Memorial Laboratory, were protected against lymphosarcoma 6-C3H-Ed by previous implantation of lymphoid tissue from a variety of random bred and pure strain mice. Spleen tissue from ZBC mice was found to be particularly active as an antigen in inducing this immunity. These studies now include experiments in which lymphoid tissue from several sublines of C3H mice were implanted in mice of other C3H sublines followed by challenge with lymphosarcoma 6-C3H-Ed, and attempts were made to broaden the findings which had been made with this tumor using similar methods and other lymphoid tumors at our disposal.

Materials and methods. Jax-C3H (He), DBA-2, C57 Black, A (Lilly), and A (He) mice were obtained from the Jackson Memorial Laboratory. ZBC mice (produced by mating mice of the A strain and Dr. Bittner's C3H (called Z-strain) to produce F_1 hybrids, and mating F_1 females with Z males) were obtained, together with a supply of C3H (called Z) mice from Dr. J. J. Bittner. C3H mice of the Andervont stock were obtained from Dr. H. B. Andervont. CF_1, CFw and AKM mice were obtained from Carworth Farms and "Manor" mice from Manor Farms. Lymphosarcoma 6-C3H-Ed, (C3H (He) mice), and lymphatic leukemia P-1534 (DBA-2 mice) were obtained from the Jackson Memorial Laboratory. The Patterson lymphosarcoma (AKM mice) was obtained from Dr. C. C. Stock, and lymphoma 2 (A (He) mice) was obtained from Dr. Emma Shelton through Dr. L. L. Law. The tumors were routinely passed in the pure strain mice designated in parentheses. Approximately 15 mg pieces of lymph nodes and of the various tumors were implanted by trocar subcutaneously on the left flank of the mice. Spleen cell suspensions were implanted subcutaneously by injection, each mouse receiving approximately 18-20 mg of moist spleen tissue in 0.25 ml of saline suspension. Seven to 10 days after these preliminary implantations, the mice were challenged subcutaneously in the right flank with approximately 5 million tumor cells suspended in 0.1 ml of saline. Similar tumor injections were made in control mice of the same strain. The mice were palpated twice weekly and observed either until death, or until it became obvious that the challenge tumor had regressed or had failed to grow, as judged by progressive tumor growth and death of the appropriate control mice. Apparently immune mice were rechallenged and observed for an additional 4 weeks before being finally classified as immune. In some instances, the mice available for study was few, but, with these exceptions, all experiments were carried out with groups of 8 mice.

Results. Implantation of lymphoid tissue from mice of certain sublines of the C3H strain results in immunity of recipient mice of other C3H sublines subsequently implanted with lymphosarcoma 6-C3H-Ed. Immunity of the mice was manifest by failure of the implanted tumor to grow at all or by regression after reaching palpable size. The results of experiments in which lymphoid tissues from various sublines of C3H mice were implanted in mice of other C3H sublines, followed by challenge with lymphosarcoma 6-C3H-Ed, are shown in Table I.

Spleen or lymph node-thymus mixtures from Bittner C3H (Z) mice regularly immunizes Jax-C3H (He) mice. Spleen from ZBC mice is also effective. Spleen from Jax-C3H (He) mice immunizes Bittner C3H (Z). Spleen from both Jax-C3H (He) and Bittner C3H (Z) mice immunizes Andervont C3H mice against lymphosarcoma 6-C3H-Ed, but spleen from Andervont mice fails to immunize either Bittner C3H (Z) or Jax-C3H (He). Spleen from ZBC does not immunize Bittner C3H (Z) mice.

Experiments were made to study the effect of various treatments of spleen cells from ZBC mice on their antigenicity in Jax-C3H (He) mice and to determine whether implantation of lymphoid tissues from alien strains would immunize ZBC mice against lymphosarcoma 6-C3H-Ed, or A (He), AKM, or DBA-2 mice against lymphoid tumors which grow progressively in these strains. Results of these experiments are shown in Table II.

It is seen in Table II that none of the tissues implanted into ZBC mice induced immunity against lymphosarcoma 6-C3H-Ed, nor did tissues from the alien strains of mice immunize DBA-2 against leukemia P-1534,

TABLE I. Immunity Produced by Lymphoid Tissues Implanted in Various Sublines of C3H Mice Against Lymphosarcoma 6-C3H-ED.

Strain and type of mouse tissue implanted	Recipient mice	No. of mice	Protected	Not protected
ZBC spleen	Jax-C3H (He)	75	73	2
Bittner C3H (Z) spleen	"	11	11	
Bittner C3H (Z) lymphnode/thymus	"	12	11	1
Andervont C3H lymphnode/thymus	"	4		4
Andervont C3H spleen	"	4		4
ZBC spleen	Bittner C3H (Z)	2		2
Jax-C3H (He) spleen	"	2	2	
Andervont C3H "	"	3		3
Jax-C3H (He) "	Andervont C3H	2	2	
Bittner C3H (Z) "	"	2	2	
Growth of tumor in controls	ZBC	300+	Progressive to death	
	Jax-C3H (He)	300+	"	
	Andervont C3H	1	"	
	Bittner C3H (Z)	4	"	

TABLE II. Attempts to Induce Immunity Against Lymphoid Tumors by Implantation of Alien Mouse Tissues.

Strain and type of mouse tissue implanted	Recipient mice	No. of mice	Tumor	Protected	Not protected
Bittner C3H (Z) spleen	ZBC	13	Lymphosarcoma (6-C3H-Ed)		13
Bittner C3H (Z) lymphnode/thymus	ZBC	8	"		8
Andervont C3H lymphnode/thymus	ZBC	4	"		4
Spleen or lymphnode from: CF_1, CFW, DBA-2, C57 Black, Manor AKM, A (Lilly), A (He) Jax-C3H (He), ZBC	ZBC	160*	"		160
"	DBA-2	160*	Leukemia—P-1534		160
Spleen from above mice	AKM	80†	Patterson lymphosarcoma		80
"	A (He)	80†	Lymphoma 2		80
ZBC spleen	Jax-C3H (He)	75	Lymphosarcoma (6-C3H-Ed)	73	2
ZBC lymphnode/thymus	"	16	"	3	13
ZBC spleen (frozen and thawed)‡	"	8	"		8
ZBC " (crushed to break cells)§	"	8	"		8
ZBC " (lyophilized)	"	8	"		8
ZBC " (boiled)	"	8	"		8
ZBC " (cells suspended in .1% formalin/saline)	"	8	"		8
ZBC spleen (fresh spleen, mice treated with cortisone acetate 40 mg/kg/day/5 days)	"	16	"		16

* Groups of 8 mice each received either spleen or lymphnode from various strains of mice.
† " " " " " " " spleen from various strains of mice.
‡ Frozen at −18°C for 2 hr, then slowly thawed at room temperature.
§ Ground with sand in saline.

AKM against the Patterson lymphosarcoma nor A (He) mice against lymphoma 2. Spleen cells of ZBC mice are much more active than lymph node/thymus mixtures in immunizing Jax-C3H-Ed mice against lymphosarcoma 6-C3H-Ed. Any treatment which ruptures the cell wall abolishes the antigenicity of ZBC spleen tissue under the conditions of these experiments, and treatment with cortisone acetate prevents the development of immunity against this tumor.

Discussion. It was previously shown that lymphoid tissue from a variety of random bred mice, and from mice of known genetic constitution would immunize Jax-C3H (He) mice against progressive growth of lymphosarcoma 6-C3H-Ed. Similar relationships are shown in the present experiments in which

lymphoid tissue from mice of several C3H sublines were implanted into mice of other sublines. Mice of the Heston, Bittner(Z), and Andervont C3H sublines, and ZBC mice, in all of which this tumor grows progressively, show different behavior in immunizability following subcutaneous implantation of spleen or lymph node and thymus tissue from other sublines of C3H mice. Tissue from Bittner C3H (Z) and Jax-C3H (He) mice reciprocally immunized against this tumor. Spleen from Andervont C3H mice did not immunize Bittner C3H (Z) mice, yet spleen from the latter, and from Jax-C3H (He) mice immunized Andervont mice. ZBC spleen failed to immunize Bittner C3H (Z) mice. Although many have been tried, none of the tissues yet implanted into ZBC mice have immunized against lymphosarcoma 6-C3H-Ed.

It is of interest that lymphoid tissue from one C3H subline should immunize mice of another subline against a tumor which grows progressively when transplanted in all C3H mice tested. It is assumed that the immunity observed has as its basis the existence of antigenic differences between the tissues of one type mouse from that of the others, and whatever these differences may be, it would appear that antigenic tissues are widely distributed, for protection of Jax-C3H mice can be accomplished not only by implantation of tissues from other C3H sublines as in the present instances, but, as was shown previously(4), spleen or lymph node and thymus tissue from random bred mice, (CF_1, CFw and Manor), and pure lines (DBA-2, C57 Black) will also induce immunity. In this connection it is important to note the apparent differences in antigenicity of spleen and lymph node thymus tissue of ZBC mice in immunizing Jax-C3H (He) mice against lymphosarcoma 6-C3H-Ed-spleen regularly induces immunity, while the lymph node thymus mixture does so infrequently.

The data suggest that genetic differences between sublines of C3H mice might be detectable by immunological tests of the type described. Such differences have been previously shown to exist between the Bittner and Andervont(6) and between the Bittner and the Strong sublines(7) by differential susceptibility of the mice to transplantable mammary carcinoma.

Implantation of intact cells appears to be a requirement for antigenicity, which is in keeping with the observations of Barrett *et al.* (3) who studied immunity produced in C strain mice injected with blood from DBA mice against a fibrosarcoma which arose in DBA mice, but which was transplantable to C mice. Development of immunity in Jax-C3H (He) mice against lymphosarcoma 6-C3H-Ed is prevented in mice treated with cortisone acetate (40 mg/kg for 5 days) beginning on the day that ZBC spleen was introduced.

In our experience, the antitumor immunity developed by implantation of lymphoid tissue from certain strains of mice can be developed only against lymphosarcoma 6-C3H-Ed. Repeated trials failed to induce immunity in DBA-2 mice against leukemia P-1534, in AKM mice against the Patterson lymphosarcoma and in A (He) mice against lymphoma 2. These results suggest a close similarity in the antigenic constitution of lymphoid tissue of certain strains of C3H and other mice(4) to that of lymphosarcoma 6-C3H-Ed.

Summary. Experiments are described in which spleen or lymph node from certain sublines of C3H mice when implanted into mice of other sublines of the C3H strain immunized them against progressive growth of subsequently implanted lymphosarcoma 6-C3H-Ed. In repeated similar trials in other strains of mice, no immunity was produced against lymphoid tumors which grow progressively in these strains.

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