Effect of Thymectomy on Development of Leukemia in C3H Mice Inoculated with Leukemic "Passage" Virus.* (24615)

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Thymectomy has been known to inhibit development of either spontaneous leukemia in Ak mice(1,2), or radiation-induced leukemia in strain C57 Black(3), or C3H(4). It appeared of interest to determine whether leukemia induced by inoculation of a transmissible, highly potent cell-free agent(5-7) could also be inhibited by removal of thymus.

Methods. Passage A strain of leukemic virus initially derived from spontaneous Ak leukemia(5), and then passed serially by cell-free inoculations of newborn mice(6,7), was used. In each experiment, filtrates (Selas 02) of 20% concentration, were prepared in the usual manner(6,7) from pooled leukemic organs removed from several individual C3H donors with primary, passage 12th to 14th, virus-induced leukemia. All mice were of the C3H, or foster nursed C3H(f) lines, both of Bittner substrain, bred in our laboratory by brother-to-sister mating. Incidence of spontaneous leukemia in untreated mice of our colonies of C3H and C3H(f) mice does not exceed 0.5%(7). Thymectomy was performed aseptically under combination of light, nembutal-ether anesthesia. After skin incision, the upper part of sternum was exposed, and the manubrium sterni split in middle line longitudinally (3 to 4 mm) with fine scissors. The fascia covering mediastinum was then gently lifted with an ophthalmic forceps, and a small opening (3 to 4 mm) made, to visualize the thymus. Both thymic lobes were then removed by gentle suction, using a small, glass tubing. The wound was closed superficially by 2 or 3 interrupted silk sutures, involving only the skin. Sufficient skill was gradually acquired to allow performing this simple operative procedure with mortality not exceeding 10 to 20%.

Results. Thymectomy in young adult C3H mice, followed by inoculation of leukemic virus. Young, adult C3H mice, approximately 4 to 6 weeks old, were divided into 2 groups. Brothers and sisters were equally divided, but not all animals were litter-mates. Mice of the experimental group were thymectomized. The other group was left untreated, as controls. After 3 to 7 days, animals of both groups were inoculated intraperitoneally (0.5 ml each) with leukemic filtrate. This experiment was based on the potency of newly developed passage A virus to induce leukemia, following inoculation into young adult C3H mice(7). It is evident from Table I that, thus far, all 39 thymectomized mice, which survived operative procedure and inoculation of virus, remained free from leukemia, whereas 44% of 41 controls, that had been inoculated simultaneously with the same filtrates, developed leukemia at

TABLE I. Thymectomy in Young Adult C3H Mice, Followed by Inoculation of Passage A Leukemic Filtrate.*

	Thymectomized mice†				Controls				
	No. of exp.	Sex	No. of mice inoc.	No. dev. leuk.	Sex	No. of mice inoc.	No. dev. leuk.	Leuk. inc., %	Avg age leuk. dev., mo
		♀	27	0	♀	27	11		
		♂	12	0	♂	14	7		
Total	3		39	0		41	18	44	7

* All filtrates (Selas 02) of 20% conc. A different filtrate was prepared in each experiment. Each mouse received 0.5 ml of filtrate i.p. Avg age at inoc., 60 days. Mice that died within 6 wk after inoculation were not included in table; this included operative mortality resulting from thymectomy.

† Time interval between thymectomy and inoculation of filtrate, 3 to 7 days.

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TABLE II. Inoculation of Passage A Leukemic Filtrate* into Suckling C3H Mice, Followed by Thymectomy.

	Thymectomized mice†					Litter-mate controls				
	No. of exp.	No. of litters inoc.	Sex	No. of mice inoc.	No. dev. leuk.	Sex	No. of mice inoc.	No. dev. leuk.	Leuk. inc., %	Avg age leuk. dev., mo
			♀	32	0	♀	39	38		
			♂	23	1	♂	39	30		
Total	20	38		55	1‡		78	68	87	2.7

* In each exp., different filtrate (20%, Selas 02) was prepared. 16 mice in thymectomized, and 18 in control group were inoculated s.c., when less than 16 hr old; all other mice in both groups inoculated i.p., when 1 to 8 days old (avg age at inoc., 3 days). Mice that died within 6 wk after inoculation were not included in table; this included immediate mortality resulting from thymectomy.

† Thymectomy was performed when inoculated mice were 3 to 6 wk old (avg age at thymectomy, 1 mo).

‡ 1 mouse developed palpable spleen at 2.7 mo of age, but is otherwise in good health.

7 months average age. Among 18 positive leukemic mice, 16 had a generalized leukemia, and in 2, on macroscopic examination, leukemia was limited to thymic lymphosarcomas. Although this experiment has not yet been terminated (average age of surviving mice 8 months at time of writing) the results thus far obtained are clear cut.

Inoculation of leukemic virus into suckling C3H mice, followed by thymectomy. C3H mice, less than 9 days old, were inoculated intraperitoneally with leukemic filtrate. After the mice reached 3 to 6 weeks of age, the litters were divided into one experimental, and a control group. Mice of the experimental group were then thymectomized (average age at thymectomy 1 month). Both groups were then observed for development of leukemia. It is evident from Table II that thymectomy inhibited development of leukemia, in essentially all animals, only 1 out of 55 mice developing a palpable spleen, and 1 developing definite leukemia. On the other hand, in the control group, 68 of 78 littermates developed leukemia (87%) thus far, mostly generalized (60) or limited on macroscopic examination to thymic lymphosarcomas (8). These experiments are still in progress (average age of surviving mice 4.5 months at time of writing); leukemia may still develop in some thymectomized mice, and will probably also develop in a few control animals. Even with these reservations, the results thus far obtained suggest inhibition of development of leukemia, following thymectomy, in mice inoculated with the leukemic agent.

Discussion. It was quite unexpected to observe that removal of thymus, either preceding, or following inoculation of leukemic passage A filtrates, inhibited development of leukemia in C3H mice. As an explanation, let us assume, as a working hypothesis, that the thymus represents the principal target organ, where the injected leukemogenic agent is stored, and multiplies. In the first series, there was no "target" organ available for the agent when the filtrate was injected, since mice had been thymectomized prior to inoculation. In the second series, removal of thymus in mice previously inoculated could have eliminated most of the virus presumably stored in that organ. The agent could have been stored in mice of both series also in other organs, such as spleen, liver, lymphoid nodes, etc. One might speculate that no disease resulted in most of such animals because a massive concentration of a potent agent may be needed in a particularly sensitive cell area, as may be available in the thymus, to assure sufficiently rapid replication, and eventual activation, of the agent. This would further imply that the initial focal activation of the disease would usually take place in the thymus, then disseminated rapidly. There is not sufficient evidence to imply, however, that all lymphatic leukemias in the mouse initially develop in the thymus. Other explanations therefore should also be considered; the role of thymus in development of leukemia may also be humoral, or otherwise activating the agent. Further studies are needed.

The second series, in which suckling C3H

mice were first inoculated with leukemic virus, and then thymectomized, could be compared with the removal of thymus in young Ak mice; the latter carry naturally the same, or a very similar agent. In the injected C3H mice, the agent is introduced, by inoculation, into the suckling host, shortly after birth. In Ak mice, the agent is already present in the embryo, having been "vertically"(5) transmitted, *i.e.* acquired from parents. Both, injected C3H, and naturally infected Ak mice, carry therefore a potentially pathogenic leukemic agent. Irrespective of theoretical explanation, in both groups of animals, removal of thymus may inhibit development of leukemia.

That the thymus of young, normal Ak mice actually contains considerable quantities of the agent was evident from unpublished experiments we carried out, in which cell-free extracts prepared from normal thymic tissue aseptically removed from 4 to 6 week healthy Ak mice, were inoculated into newborn mice of the C3H strain, and induced leukemia. Other normal Ak organs, such as testes and ovaries, however, also carried the agent(8) suggesting that thymus is quite probably not the exclusive target area for storage of leukemic virus in the mouse.

It is true that C3H mice without injection of a leukemic virus will often develop leukemia following total body x-ray irradiation(4); this radiation-induced leukemia is apparently also caused by a transmissible virus, since it can be transmitted by filtrates(4,9,10). The agent apparently responsible for development of radiation-induced leukemia is of low potency, remaining latent, unless triggered by total body irradiation. This x-ray induced agent is apparently also stored in the thymus, since radiation-leukemia can also be prevented by thymectomy(3,4). Whether the Ak agent, causing leukemia following inoculation into C3H mice is identical with the latent agent, indigenous in normal C3H mice, and causing radiation-induced leukemia, remains to be determined; we believe that these are 2 distinct, though possibly related, leukemogenic agents. Whether acquired by inoculation, or by "vertical" transmission from host's parents, both are apparently stored principally in the thymus.

Thus, C3H mice in which thymus was removed during their first two months of life were resistant to leukemogenic action of either total body x-ray irradiation(4), or of transmissible virus known to induce otherwise leukemia in 90% of normal mice of the same inbred line(5-7).

Our results present a rather unusual example of preventing development of a transmissible, virus-caused disease, by surgical removal of a target organ, in an otherwise susceptible host.

It should be stressed, however, that although removal of thymus may have an inhibiting action on development of primary, virus-induced leukemia, it does not influence development of leukemia resulting from implantation of leukemic cells into a susceptible host. This was shown before(11) in Ak mice injected with Ak leukemic cells, and also observed in our laboratory on thymectomized young adult C3H mice inoculated with C3H leukemic cells (unpublished).

Summary. 1. Of 39 young and adult C3H mice, thymectomized, and a few days later inoculated intraperitoneally with passage A leukemic filtrate, none developed leukemia. Of 41 normal controls inoculated simultaneously with the same filtrate 18 (44%), developed leukemia at 7 months average age. 2. Of 55 suckling C3H mice inoculated with passage A leukemic filtrate when less than 9 days old, then 1 month later thymectomized, only 1 thus far developed a palpable spleen, and definite leukemia. Of 78 control litter mates inoculated simultaneously with the same filtrates, but not thymectomized, 68 (87%), developed leukemia at 2.7 months average age. 3. Thymectomy, either preceding, or following inoculation of leukemic passage A filtrate, inhibits development of leukemia in otherwise susceptible C3H mice.

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Inactivation by Plasma of ACTH-Releasing Property of C^{14} Labeled Bacterial Polysaccharides.*† (24616)

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A marked, but transient, elevation of plasma 17-hydroxycorticosteroids (17-OHCS) follows intravenous injection of a polysaccharide complex from *K. pneumoniae* into the guinea pig(1.2). The plasma 17-OHCS return to control, while abundant C^{14} labeled polysaccharide remains in blood plasma(2). During the first few hours after injection, the bacterial polysaccharide progressively loses capacity to evoke a 17-OHCS response(2). Such observations suggest that a physicochemical change may have occurred in the polysaccharide after its administration. It has been demonstrated that the polysaccharide will not deplete adrenal ascorbic acid or decrease adrenal cholesterol in hypophysectomized rats(3). Further, Rosenfeld(4) was unable to increase *in vitro* production of adrenal steroids by perfusing isolated adrenal glands with different amounts of polysaccharides. Hypercorticism following administration of polysaccharide must, therefore, be due to increased secretion of ACTH by the anterior pituitary gland. The present investigation concerns *in vivo* and *in vitro* binding of C^{14} labeled polysaccharide with plasma proteins in relation to ACTH releasing capacity and the haptenic property of the polysaccharide.

Materials and methods. The preparation of C^{14} labeled (alkaline-extracted, haptenic, non-lethal) polysaccharide complex from *K. pneumoniae* has been described(5). The haptenic quality of isotopically labeled polysaccharide in circulating plasma was evaluated by quantitative precipitin technic using anti-*Klebsiella* rabbit plasma inactivated at 56°C. The original labeled polysaccharide was employed as a reference standard. The optimal range of the precipitin reaction was 1 to 5 γ/ml of rabbit plasma. Controls consisted of polysaccharide in 0.85% NaCl with normal rabbit plasma, polysaccharide in circulating guinea pig plasma with normal rabbit plasma and polysaccharide-containing plasma with 0.85% NaCl. Guinea pigs of either sex, weighing 200 to 250 g were given a single injection by ear vein with 20 γ C^{14} labeled bacterial polysaccharide/100 g body weight. The labeled polysaccharide was given in 0.15 M NaCl, 0.1 ml/100 g body weight, and a control group was given 0.1 ml 0.15 M NaCl/100 g body weight. Animals in both groups were killed ½, 1, 3, and 6 hours afterwards, employing cervical clamp technic(2). The heparinized plasma obtained by cardiac puncture was immediately centrifuged and plasma removed. Plasma 17-OHCS were determined by the method of Eik-Nes(6). Previously we isolated cortisol in guinea pig plasma(4) and plasma levels of 17-OHCS in the guinea pig apparently reflect the concentration of cortisol in this fluid. Following cervical clamp technic(2), plasma levels of 17-OHCS in normal guinea pigs will range from 1 to 30 γ/100 ml with mean concentration of 21 γ/100 ml

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